Integrally Evaluation of production safety and genotoxicity of recycling residual sludge for drinking water treatment plants

Meng Yao
Shenzhen Institute of Information Technology
ting chen
Guangdong GDH water company limited
zhilin ran (zhilinran_sziit@163.com)
shenzhen institute of information technology https://orcid.org/0000-0003-4615-1587
qibao wu
Shenzhen Institute of Information Technology
shaofeng Li
Shenzhen Polytechnic institute

Research

Keywords: Recycling residual sludge; Drinking water treatment plant; gene mutation assay

Posted Date: November 22nd, 2019

DOI: https://doi.org/10.21203/rs.2.17610/v1

Abstract

Background: Recycling residual sludge in Drinking Water Treatment Plants could provide numerous benefits, like enhancing pollutant treatment efficiency and saving coagulant dosage. However, residual sludge may release excessive heavy metals and organic matter, some of which are of concern for their toxic and carcinogenic potential. The study was to investigate potential genotoxic, cytotoxic and mutagenic effects of recycled residual sludge to quality of finished water in potable water works. Methods: Based on different biotrophic level, species and genetic end point, multiple test animals and different target cells were screening out to establish a relatively systematic and comprehensive evaluation system of potential genotoxic events. Genotoxic effects of reusing residual sludge were evaluated using a combination of seven different bioassays: Ames test, Sperm abnormality test in mice, micronucleus assay (Zebrafish cell, Mice bone marrow cells and Hamster ovary cells), Comet assay and Single cell gel electrophoresis assay were separately applied to detect gene mutation, chromosome aberration and DAN damage. Result: The results of Ames assay show that the disinfected water sample display bacteriostasis at dose of 7 L/dish regardless of treatment styles, but MR<2 can still be judged as negative. The micronucleus rates of conventional treatment were slightly genotoxic but only at 4 and 40 L/kg·bw, while micronucleus rates of filtered water and disinfectant from recycling process were negative in all dose groups. The levels of DNA damage caused by different treatment processes were substantially equivalent. Conclusion: Reusing residual sludge for DWTP did not contribute to the release of genotoxic or mutagenic compounds, while had remarkable effect on saving drug dose and increasing drinking water yield, which should be widely recommended.

Introduction

As we all known, the most part of residual sludge from Drinking Water Treatment Plants (DWTP) was directly discharged into surface waters, stored in deep lakes and oceans.(Wang 1992) Residue sludge gradually becomes an inevitable byproduct largely generated during potable water production and its disposal has long been dumped landfills, which entails high costs and availability of land needed. More than 70% DWTPs have still employed coagulation-sedimentation-filtration-disinfection in their flow schemes for water purification. Unfortunately, coagulation-sedimentation imposes a high coagulant usage as well as treatment and disposal of a large volume of residual sludge. (Moghaddam SS 2011)

Residual sludge inevitably contains high concentrations of Al and Fe because of the use of Al/Fe salts as coagulants for water purification. (Nair and Ahammed 2015) Al and Fe in residual sludge mainly consisted in amorphous phases, endowing porosity and high adsorption capacity.(Ippolito 2011) Based on these properties, residual sludge from DWTP could be viewed as a valuable raw material for contaminant remediation in waste water. (Sadri Moghaddam et al. 2010) Residual sludge was applied as an effective adsorbent of many contaminants, such as P(Makris et al. 2004), hydrogen sulfide(Wang and Pei 2012) ,heavy metals (Lin et al. 2017) and toxic organic substance(Punamiya et al. 2013). Besides, some previous researchers investigated that alum-based sludge contains a large portion of insoluble alum hydroxides, which could be used as a practical solution to remove organic and inorganic materials. Qi et al has stated that alum sludge recycled from water and wastewater could not only enhance the removal efficiency of natural organic matter of a primary sewage treatment but also alleviate the load of sludge treatment and disposal in water treatment works.(Qi et al. 2009) In our former research, we found recycled alum sludge could be used as a coagulant agent with poly aluminum chloride (PACl) and aluminum sulfate ($Al_2(SO_4)_3$) to improve the removal of residual turbidity and humic acid.(Xu et al. 2016a) Jangkorn et al evaluated the feasibility of reusing the aluminum sulfate sludge as a coagulant for the treatment of industrial wastewater and found that the removal efficiency of total suspended solids(TSS), total chemical oxygen demand (TCOD) and total anionic surfactants were increased.(Jangkorn et al. 2011) Therefore, successful recycling of residual sludge can be beneficial to both environment and economy.

However, beneficial and successful reuse of residual sludge of DWTP should be basis of the comprehensive understanding of potential pollution risks. Normally, residual sludge was considered non-hazardous, and this conventional point of view has been confirmed by previous study.(Ippolito 2011) These studies indicated that although residual sludge contain various metal ions of Al, Fe, As, Cu, Mn and Zn, most metals tend to occur in low concentrations and exhibit relatively stable forms. (Wang et al. 2014a, Wang et al. 2014b, c) The potential risks presented by metals in residual sludge have been evaluated in the remediation of sediments or constructed wetlands.(Babatunde and Zhao 2010, Yuan et al. 2016b) This is to say, application of residual sludge could typically enhance removal efficiency of contamination, whereas little reports have clearly determined the toxic effect of residual sludge of DWPT on water treatment. (Misik et al. 2011)

Traditional parameters cannot guarantee the safety of recycled sludge in DWTPs, because some toxics, which are not detected by DWTPs, may accumulate during the circulation process and cause potential health risks. A combination of genotoxicity test can give a more comprehensive and accurate conclusion, which is necessary and important.In this context, the aim of this present work is to investigate the genotoxic and mutagenic properties of Municipal Drinking Water Treatment Plants effluent with reusing residual sludge, especially established a systematic toxicology evaluation system. A combination of genotoxicity tests affecting different endpoints (genetic mutations, chromosomal mutation, and DAN damage) in different cells was performed.The key content of this study is to select several test

combinations from many testing methods and simultaneously judge the real toxicity of the tested substance more accurately. Mammalian cells and vivo tests were selected to provide the highest predictive value for detecting effects that are relevant for humans, which are complementary for different classes of environmentally relevant DNA carcinogens and endpoints.

Materials And Methods

2.1 Experimental setup and procedure

A pilot coagulation-sedimentation-filtration-disinfection setup was used in this experiment to investigate the conventional and recycling drinking water treatment process. The sketch and design parameters could be referred in our former research. (Chen et al. 2015) Source water originated from Nenjiang River was obtained from the intake of Zhongyin water treatment plant (Daqing, China), which was characterized as typical slightly polluted surface water with low turbidity and color. Waste residual sludge was collected from sedimentation tank and filter backwash water respectively, which was stored in two custom-built sludge storage tanks and pumped to the head of the static pipeline mixer after being completely mixed. Characteristics of raw water and residual sludge were shown in Table 1. Sludge from different treatment cell has higher particle density and organic matter concentration, especially for value of turbidity and UV_{254}. Additionally, polyferric aluminum chloride (PFAC) was used in coagulation cell. It was content of 8.1% Fe_2O_3 and 3.3% Al_2O_3, basicity 6.2%. In other words, release of metal ions during recycling process could aggravated water quality risk.

Table 1 The water quality parameter of waste water in water supply plant

Contaminant indicator	Raw water	Sludge from sedimentation tank	Filter backwash water
Turbidity（NTU）	4-7	500-2560	250-460
Color(CU)	28-35	350-420	85-160
TOC (mg/L)	4.4-5.6	4.85-7.2	4.61-5.32
DOC (mg/L)	3.85-4.65	4.70-6.01	4.53-5.16
UV_{254}（cm^{-1}）	0.065-0.076	0.091-0.298	0.072-0.081
NH_3-N (mg/L)	0.348-0.522	0.412-0.590	0.301-0.443
Al (mg/L)	0.316-0.484	0.124-0.319	＜0.050
Fe (mg/L)	0.176-0.321	0.023-0.048	＜0.010
Mn (mg/L)	0.027-0.054	0.080-0.135	0.023-0.036

2.2 Collection and concentration of Water samples

200-L water samples were taken from all sampling points (shown in abbreviation lists), immediately acidified with hydrochloric acid to pH=2, and then concentrated over an Amberlite XAD-2 resin (Sigma) column (2.2*23), followed by water sample filtration at a rate of 30–40 mL/min. The resins were pre-treated by consecutive 8-h soxhlet extractions with methanol, dichloromethane, hexane and acetone and then stored in methanol at 4 °C prior to use. The elution of the adsorbed organics from the resin column was done with 350 ml of hexane and acetone (hexane: acetone=17:3) and 560 ml of dichloromethane. The extracts were evaporated to dryness with a rotary vacuum evaporator and then dissolved in 2 ml (dimethylsulfoxide) of DMSO (0.025%) and stored at 20 °C until a bioassay test. The extract concentrations were expressed as to 100 L of water per 1 ml of DMSO. The DMSO was applied as negative control in the percent of 0.025. (Chen et al. 2016)

2.3 Physico-chemical analysis

Turbidity was measured with a turbidimeter (HACH2100P, Hach Company, USA) according to US EPA method #180.1. DOC and TOC were analyzed by a TOC analyzer (TOC-VCHP, Shimadzu Corporation, Japan). The UV absorbance at 254 nm (UV_{254}) was determined using a spectrometer (DR5000 UV/VIS, Hach Company, USA). Both DOC and UV_{254} were measured after filtration through 0.45 μm membranes. The THMFP was also determined, as the parameter estimates the expected concentration of THMs in water samples of conventional and recycling processes with an excess of free chlorine (APHA 2012)

2.4 Mutagenitcity test: Salmonella/microsome assay (Ames test)

The tests were performed according to standard incubation method (APHA 2012), and the details were shown in supplementary 1.

2.5 Chromosome aberration tests

According to different species, 4 different experiments were selected to investigate chromosome aberration. The test methods were shown in supplementary 2-5.

2.6 Alkaline single-cell microgel-electrophoresis assay (Comet assay)

The tests were performed according to standard incubation method (APHA 2012), and the details were shown in supplementary 6.

Results

3.1 Optimal recycling condition

In our previous research, different combination of reflux ratio of backwash water and sedimentation tank sludge could significantly influence the coagulant effect.(Xu et al. 2016b) Therefore, filter backwash water and sediment discharge water were set as different recycling rate to achieve optimal save reagents rate, and results showed as Fig. 1(a). The recycling ratio of filter backwash water were set as 4%, 5% and 6%, while recycling ratio of sedimentation tank sludge were set as 2% ,3% and 4%, respectively. The results indicated that reuse ratio of filter backwash water and sedimentation sludge water in different proportion will have different effects on reagent saving rate. The recycling combination of 4%+2%, 4%+3% and 4%+4% showed save rate was negative, as range of -20%～-17%, which means can not reduce dosage of coagulant. Save rate of zero at the combination of 6%+2% stated that dosage of coagulant was as same as the conventional treatment. Under the condition residual turbidity (less than 2 ± 0.5NTU) meets the standard, and combination 6%+4% has irreplaceable advantages. Therefore, this combination was selected for the subsequent experiments.

Mixed turbidity of filter backwash water and sedimentation tank sludge is the key control parameter to realize water and reagent saving in drinking water treatment.(Xu et al. 2016a) The effect of mixed wastewater turbidity on coagulant saving rate was shown in Fig. 1(b). There was optimal range of mixed wastewater turbidity (125-175 NTU) when simultaneously recycled both filter backwash water and sedimentation tank sludge, accompanied by save reagent rate was 13%-30.5%. The residual particulate matter existed in recycled water could increase the collision and sticking probability to optimize coagulation process. On the premise of water quality meets the stander, the quantity of production waste water is saved, the dosage of reagent is saved, and the operation cost is effectively reduced.

3.2 Evaluation of water quality safety with regular water quality indicators

In order to evaluate water quality safety between recycling and traditional process,

regular water quality indicators including turbidity value, TOC, DOC, UV_{254}, Al, Fe, Mn and THMFPs were continuously monitored for 15 days, and results were illustrated in Fig.2. The change range of turbidity (shown in Fig. 2a) was unconspicuous means turbidity was not increased during recycling process probably because large particles in recycled wastewater promoted coagulation effect. The detection results of three different metal ions were shown in Fig. 2 (b-d). The variation range of aluminum ion in different treatments were tend to be consistent, and the average values were 0.152 mg/L and 0.131mg/L respectively. The Ferric ion concentration of recycling cell were lower than that of conventional treatment, while the manganese ion content was basically same. As a result, recycled production wastewater could not induce accumulation of metal ion.

It is known that organics pose a potential threat to human health and are difficult to remove by the conventional treatment process. (Nair and Ahammed 2015) During continuous operation for 15 days, variation trend of TOC, DOC and UV_{254} were shown in Fig.2(e-g). The average values of TOC, DOC and UV_{254} were respectively 4.54 and 4.68mg/L, 4.32 and 4.1 mg/L and 0.067 and 0.062cm^{-1} in conventional and recycling treatment. The little differences and smooth fluctuation mean directly reused production waste water could not result in the increase of organic matter. Average value of THMFPs (Fig. 2h) was lower than that of conventional process with about 6.8 μg/L, indicated that reflux process is more conducive to remove dissolved organics and trihalomethane precursors. Therefore, recycling treatment has obviously advantages from the perspective of water quality safety.

3.3 Evaluation of water genetic toxicity

3.3.1 Mutagenitcity test: Salmonella/microsome assay (Ames test)

The Ames tests were conducted to investigate the effect of water extracts from conventional and recycling treatment on gene mutation. The Salmonella/microsome assay results, expressed as MR, are presented in table 2.

Table 2 The Ames experiment results of various filtered water and disinfection effluent

Sample	Before disinfection in recycling				After disinfection in recycling				Before disinfection in convention				After disinfection in convention				Revertant	Negative control	Positive control
dosage（L/plate）	7	5	3	1	7	5	3	1	7	5	3	1	7	5	3	1			
TA98	28±9	34±19	40±24	38±27	4±5e	32±26	39±12	28±4	26±10	26±14	33±9	30±10	8±5e	35±21	56±40	34±9	37±5	32±4	1188±158*
MRTA98a	0.76	0.92	1.08	1.03	0.11	0.86	1.05	0.76	0.7	0.7	0.89	0.81	0.22	0.95	1.51	0.92	—	0.86	32.11*
TA100	55±18e	56±14e	71±19	92±17	0.2±0.4e	44±18e	47±16e	109±9	56±22e	60±10e	57±20e	73±9	10±5e	72±21	116±14	115±20	121±5	123±10	989±51*
MRTA100a	0.45	0.46	0.59	0.76	0	0.36	0.39	0.9	0.46	0.5	0.47	0.6	0.08	0.6	0.96	0.95	—	1.02	8.17*

Note：Mutagenicity ratio (MR): obtained dividing the revertants of sample by the spontaneous mutation rate; “*”, MR≥2, noted positive control; MR<2, not noted negative controls; “e”: bacteriostat.

According to the twofold rule (MR > 2) for positive results, all water extracts, tested at doses from 1 to 7 Leq/plate in strains TA98 and TA100 displayed negative results, regardless treatment styles. Samples at 7 leq/plate showed bacteriostasis in strains TA98 (tend to mutation frequencies) after disinfection. However, MR values were in respective of 0.11 and 0.22 in conventional and recycled process, which could be thought as negative control. As for other doses in strains TA98, MR values were all lower than two in the two treatments, meaning no toxic to bacteria. Although most of the sample at higher doses (5-7 leq/plate) were bacteriostasis whether disinfection or treatment style in strains TA100, all values of MR were less than two.

3.3.2 Sperm abnormality test in mice

The sperm abnormality test in mice was regarded as a standard in vivo assay to detect mutagenesis of the subject due to the high sensitivity to the known germ-mutagenesis. (Hallak 2017) In terms of composition ratio of various sperm malformations, the mutation was mainly folding and curling of tail, followed by abnormal change of amorphous head. The filtration and disinfection effluent were 160 L from recycled and conventional treatment, respectively, and concentrated by XAD-2 resin to proceed sperm deformity test in mice. The tested results were shown in Table 3. Compared recycled and conventional treatment, both outlet from filtration did not increase the deformity rate of mice sperm at dosage of 0.4L/kg.bw (equivalent to the human daily water requirement). When the mice were fed with 10 times the human daily water requirement, there was no mutating effect on male germ cells. However, the disinfectant water sample (R3) was observed sperm aberration at 40 L/kg.bw and malformation rate (2.02 0.54) was more than twice that of negative control group (0.79 0.24). When mice were fed with 100 times of daily water demand, the disinfection water sample after continuous reuse of waste water caused interference of normal male reproductive system with high dose treatment, but might not be permanent dysfunction in low dose treatment

Table.3 Mouse sperm abnormality test results of filtered water and disinfection effluent

Sample	C2		C3			R2			R3			Negative control	Positive control
Dosage（L/kg·bw）	40	4	0.4	40	4	0.4	40	4	0.4	40	4	0.4		
Animal numbers	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Sperm count tested	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000
Abnormal sperm count	202	195	200	204	220	170	172	184	175	504	180	231	198	938
Deformity rate（x±s）（%）	0.81±0.23	0.78±0.23	0.80±0.24	0.82±0.10	0.88±0.19	0.68±0.15	0.69±0.13	0.74±0.05	0.79±0.24	**2.02±0.54****	0.72±0.14	0.92±0.09	0.79±0.24	**3.75±0.21****

Note: **: compared with the negative control group, the rate of sperm malformation in the mouse group was at least 2 times higher than that in the negative control group.

3.3.4 Zebrafish peripheral blood Micronucleus (MN) assay

Water samples collected from every unit of the recycling and conventional process at the fifth、tenth and fifteenth day were evaluated by MN assay, and results showed in Fig. 3. The MN frequency of all water samples was significantly higher than that of NC, which suggested that both raw water and treated water could contain toxic substance induced chromosomal aberration of zebrafish peripheral blood cell. Furthermore, effluent from sedimentation and filtration regardless treatment style showed lower MN frequency than that of raw water, while expressed higher MN frequency after disinfection. Results illustrated that flocculation and sedimentation could effectively reduce genetic toxicity, because flocs have adsorption capacity to remove natural organic matter. After statistical analysis, there was no significant statistic difference between C1 and R1, C2 and R2, and C3 and R3 ($p>0.5$), which also indicated that effluent toxicity of recycling process was equivalent to that of conventional process.

3.3.5 Micronucleus test of polychromatic erythrocytes in mouse bone marrow

Mammalian cell micronucleus test is an effective method to detect gene mutations in animal cells. (Elangovan et al. 2006)The micronucleus test of mammalian cells was carried out in the effluent of conventional process and re-used process, respectively. The results were shown in table 4. The filtered water sample after conventional treatment (C2) was negative in all dose groups. However, disinfectant water (C3) was positive in medium and high dose groups (4L/kg·bw and 40L/kg·bw). The micronucleus rates of male mice were 6.0± 1.0‰ and 11.0 ±1.6‰, and female mice were 6.0± 1.6‰ and 12.4 ±2.1‰, respectively. On the contrary, the filtered and disinfectant water from recycling process were negative in all the dose groups(C3 and R3). There was no significant difference in C2 and R2 of filtered water between the two processes by $p > 0.05$.

Table 4 Micronucleus rate in mouse marrow cells of filtered water and disinfection effluent

Samples	Dosage（L/kg·bw）	Animal number	PCE number	Contains microkernel PCE number	Micronucleus cell rate（‰）
C2	40	5	5000	7	1.4±0.9
	4	5	5000	8	1.6±0.9
	0.4	5	5000	8	1.6±0.5
	40	5	5000	9	1.8±0.8
	4	5	5000	8	1.6±0.8
	0.4	5	5000	9	1.8±0.8
C3	40	5	5000	55	11.0±1.6**
	4	5	5000	30	6.0±1.0*
	0.4	5	5000	16	3.2±1.3
	40	5	5000	62	12.4±2.1**
	4	5	5000	30	6.0±1.6*
	0.4	5	5000	12	2.4±1.1
R2	40	5	5000	6	1.2±0.4
	4	5	5000	7	1.4±0.5
	0.4	5	5000	5	1.0±0.7
	40	5	5000	9	1.8
	4	5	5000	8	1.6
	0.4	5	5000	8	1.6
R3	40	5	5000	10	2.0±1.0
	4	5	5000	7	1.4±0.5
	0.4	5	5000	7	1.4±0.9
	40	5	5000	12	2.4±1.1
	4	5	5000	11	2.2±0.8
	0.4	5	5000	9	1.8±0.8
Negative control		5	5000	10	2.0±0.7
		5	5000	13	2.6±0.5
Positive control		5	5000	108	32.8±5.4**
		5	5000	169	33.8±4.7**

Note：compared with negative control：*：P<0.05；**：P<0.01；

3.3.6 Micronucleus test of CHO-K1

CHO-K1 micronucleus test can be used to characterize the chromosomal damage effect of cells. The determination condition of positive results was: when acute toxicity <50%, MN‰ presented a dose-response relationship with the exposure concentration of water sample. (Bernardi et al. 2014) In this study, one-way ANOVA test was used to verify the micronucleus rate, and significance level was p=0.05. The micronucleus rate and subdiploid rate were used to analyze the MOA generated by micronucleus. The initial water exposure concentrations were 0.5mg/L, 0.25mg/L, 0.125mg/L, 0.0625mg/L, 0.03125mg/L and 0.015625mg/L. According to the results of acute toxicity experiment, it was found that the sample exposure concentration of 0.5mg/L, the acute cytotoxicity of cell was exceeded 50%. Therefore, the micronucleus rate and subdiploidy rate were observed at the gradients of 0.25mg/L, 0.125mg/L and 0.0625mg/L. The results were shown in table 5.

Table 5 CHO-K1 test results of filtered water and disinfection effluent

Sample	Dilution ratio/Concentration(mg/L)	Micronucleus rate		Subdiploidy		Mode of action[b]
		MN(‰)	PI	MN(‰)	PI	
Solvent comparison	0	2.23	1	3.40	1	
R2	0.0625	3.18	1.43	2.40	0.71	No damage effect
	0.125	3.31	1.48	2.29	0.68	
	0.25	2.80	1.25	10.33	3.04	
R3	0.0625	2.05	0.92	22.43	6.60	No damage effect
	0.125	2.84	1.28	12.18	3.58	
	0.25	3.12	1.40[a]	14.06	4.14	
C2	0.0625	3.36	1.51	1.62	0.48	No damage effect
	0.125	4.23	1.90	2.10	0.62	
	0.25	1.18	0.53	4.93	1.45	
C3	0.0625	2.64	1.18	3.03	0.89	No damage effect
	0.125	2.83	1.27[a]	2.41	0.71	
	0.25	2.96	1.33[a]	1.64	0.48	
Mitomycin C	2.5	7.69	3.45	5.90	1.73	Chromosome rupture
Colchicine	0.8	9.39	4.22	17.11	5.03	Aneuploidy

Note: [a]:compared with control group, the micronucleus rate was significantly increased.（One-Way ANOVA，$p<0.05$）

As for filtered water samples from two different processes (R2 and C2), there was no significant difference between the micronucleus rate and the negative control, means no chromosome damage effect. Results showed that there were no genotoxic substances for chromosome damage in different filtered water. The disinfectant water samples from two different processes (R3 and C3) were significantly different from the negative control (One-Way ANOVA，$p<0.05$). Nevertheless, no chromosome damage effect was observed in all water samples. This may be because of the disinfectant water sample in the production of some damage to chromosomes caused by genetic toxicity, while extremely low levels of genotoxic substances do not cause chromosome damage. The effluent water quality difference of two water treatment processes was also evaluated from the perspective of micronucleus rate. According to the statistical principle, there was no significant difference between R2 and C2, indicating that the effluent quality after the two processes was basically the same. Similarly, the disinfection water samples of the two processes showed basically the same trend as the filtered water.

3.3.6 Comet Assay in Peripheral Blood Cells of Zebrafish

The induction of DNA single-strand breaks by SCGE assay is the most frequently recommended and employed endpoint for detecting DNA damage. (Vasiljevic et al. 2016) The SCGE assay of peripheral blood cells from zebrafish were conducted after continuous running 5, 10 and 15 days for conventional and recycling treatment, respectively. Significance differences analysis was used as criterion. The value of p larger than 0.05 was regarded as no significant difference. Statistical results were shown in Fig. 4. Raw water and effluent from all treatment, whether recycled or not, did not induce DNA strand breaks, showing tail length values similar to those of negative controls. However, the tailing intensity of filtrated water with all treatments were two to three times lower than that of raw water. The possible reason may that organic matter was wrapped by flocs after sedimentation and further intercepted through filtration process, which caused tail intensity decrease.

Table 6 demonstrated DNA damage score for drinking water sample at different points of treatment unit between conventional and recycling processes. According to the damage degree of tail length and head length, DNA damage can be divided into 0,1,2,3 and 4 levels (From no damage to complete damage). The damage degree of 100 cells was range from 0 to 400. Any unit of AU was used to indicate the extent of DNA damage. The higher the value of AU, the more serious the damage. (Zhong et al. 2001) The calculation formula is: (see Formula 1 in the Supplementary Files)

Based on the results, both raw water and R4 can cause DNA damage, and there are significant differences with blank contrast ($p < 0.05$) marked red. DNA damage was reduced after filtration by both recycling and conventional processes, but significantly increased after the water samples disinfected. This result is consistent with the comet test results. There was no significant difference between recycling process and the corresponding conventional treatment process ($P > 0.05$), suggesting DNA damage caused by the two processes was equivalent.

Table 6 DNA damage score for drinking water sample at different points of treatment unit between conventional and recycling processes

Reflux time（day）	Sampling point	Cell damage grading/%					Degree of DAN damage/AU
		0	1	2	3	4	
Blank	NC	95.6±2.1	1.2±0.31	0±0	0±0	0±0	1.2±0.31*
control	PC	0.5±0.34	2.8±0.52	4.3±0.21	6.8±0.42	85.6±0.05	374.2±0.3*
	Raw water	85.38±0.14	8.51±1.1	3.76±0.54	1.43±0.35	0.92±0.06	24.8±2.01*
	C1	87.02±0.06	7.32±1.12	3.29±0.31	1.87±0.42	0.46±1.01	21.3±1.26*a
5th day	C2	88.81±0.47	6.58±1.5	3.87±1.17	0.22±0.33	0.52±0.04	17.76±1.28*b
	C3	86.72±1.13	7.23±0.83	4.08±1.02	1.29±0.06	0.68±2.2	21.98±3.31*c
	R1	86.37±1.74	7.66±0.43	3.38±0.01	1.98±1.73	0.61±0.22	22.8±1.23*a
	R2	89.23±0.56	5.87±2.02	3.99±0.03	0.34±1.07	0.57±0.4	17.15±2.34*b
	R3	87.38±2.11	7.04±0.55	3.66±0.65	1.49±1.47	0.43±0.04	20.55c±1.73*c
	R4	83.58±2.03	10.03±0.73	4.17±1.02	1.52±0.82	0.7±0.04	25.73±2.24*
	Raw water	90.54±1.73	5.63±0.04	2.11±2.53	0.97±0.02	0.75±1.11	15.76±1.61*
	C1	91.41±1.89	4.98±0.43	1.95±1.06	1.08±0.16	0.58±0.37	14.44±2.07*d
	C2	92.56±0.38	4.31±1.11	1.66±0.28	0.98±1.63	0.49±0.33	12.53±1.32*e
10th day	C3	92.18±1.23	4.71±0.37	1.54±2.02	1.14±0.72	0.43±0.38	12.93±1.87*f
	R1	91.76±0.08	5.1±2.11	1.53±0.47	1.12±1.07	0.49±1.55	13.48±3.02*d
	R2	92.37±1.83	4.41±0.54	1.53±0.06	1.16±2.07	0.53±0.48	13.07±2.59*e
	R3	92.56±0.51	4.82±3.05	0.97±0.06	1.21±1.08	0.44±0.65	12.15±2.38*f
	R4	87.11±1.01	6.54±0.33	3.20±1.48	2.03±0.53	1.12±0.05	23.51±1.56*
	Raw water	87.58±1.44	7.03±2.04	3.76±1.23	0.62±0.08	1.01±2.22	20.45±1.03*
	C1	89.32±0.38	6.26±2.31	2.78±1.37	0.96±1.05	0.68±2.73	17.42±2.22*g
	C2	90.41±1.32	5.73±2.02	2.52±0.87	0.83±1.45	0.51±0.33	15.3±1.43*h
	C3	90.32±2.53	6.11±3.35	2.13±0.08	0.90±1.03	0.54±0.11	15.23±2.02*i
	R1	89.45±1.58	6.37±0.85	2.76±0.32	0.89±0.07	0.53±0.3	16.68±2.39*g
15th day	R2	89.86±0.38	6.12±0.26	2.66±1.03	0.74±0.53	0.62±1.04	16.14±1.58*h
	R3	90.35±2.05	6.05±0.04	2.22±1.32	0.8±0.47	0.58±0.03	15.01±2.01*i
	R4	86.09±2.22	6.49±1.63	3.84±0.83	2.36±1.02	1.22±0.68	26.13±1.11*

Note: *=p0.05,significant difference compared with blank test; Characters(a~i) =p0.05, no significant difference between water samples at the sampling points corresponding to each treatment unit of the two processes.

Discussion

This study integrally assessed the production safety and genotoxic effects of recycling residual sludge for drinking water treatment plants. Mixed water in a certain proportion could achieve a high drug saving effect. Compared with conditional treatment, regular test indicators (as turbidity, metal ions, organic matter and THMFPs) did not cumulative release, but the by-products of disinfection slightly decreased. Drinking-water treatment sludge could be applied as an adsorbent for heavy metal ion. However, there was no study evaluate the reusing treatment with toxicological methods as we knew, thus failed to be popularized in practical process. In order to provide more comprehensive evaluation of potential risks associated with the reuse of residual sludge to potable water, a set of toxicological tests covering different biotrophic level, different tested species and different genetic end points were performed.

All the toxicology results indicated that recycling residual sludge technique does not induce toxic, genotoxic, or mutagenic effects under the lower concentrated ratio. The Ames test confirmed that absence of mutagenic effects for frameshift mutations (TA98) and base-pair substitutions (TA100), whatever treatment style. (Table 2) Slight toxicity appeared at dose of 7L/plate because of disinfection, regardless of treatment strategy. The concentrated samples from recycled treatment lacked direct mutagenic metabolites. Disinfection effluent from different treatments (C3, R3 in table 3) were also proceeded sperm deformity test in mice after concentrated. Liviac et al stated that chlorination would cause an increase of water toxicity. (Liviac et al. 2011) Although the rate of sperm malformation slightly increased at all

dosage after disinfection, there were no significant difference with negative control group. There was no mutagenesis effect on male germ cells caused by disinfection after filtration in residual sludge reusing system.

The micronucleus rate indicates the toxicity degree of cells to evaluate the toxicity of water samples. Different tested animals were selected for micronucleus experiments. Shi et al indicated that higher values of MN frequency after disinfection strongly owing to some genotoxic disinfectants produced. (Shi et al. 2009) However, our results in Zebrafish peripheral blood Micronucleus (MN) assay (Fig.3) showed that there was no accumulation of toxicity in recycling of production waste water after disinfection treatment. Micronucleus test of polychromatic erythrocytes in mouse bone marrow was shown same results.(Table 4) With the increase of the reagent dose, the rate of micronucleus increased indicating there was a dose-response relationship. The micronucleus test was positive in medium and high dose after disinfection in conventional treatment. (Table 4) This finding support observation for earlier study, which stated disinfection process may cause some genetic damage. (Crebelli et al. 2005) Our results further indicated that reused residual sludge did not increase the rate of cell micronucleus, but to some extent enhanced the removal of precursors of disinfection by-products that caused cell mutations. Taken together, the vitro micronucleus test results suggested that continuous reuse of waste water will not cause the increase and enrichment of genotoxic substances induced chromosomes damage. Furthermore, continuous reuse of production wastewater is safe and has no significant difference from conventional treatment process from the perspective of chromosome damage.The negative results were further confirmed by the SCGE study, which increase the sensitivity of the combination tests, thus reducing the risk of false negatives. Water samples after disinfection significantly increased DNA damage degree in both treatment processes. The results were consistent with that of comet experiment, might reliably predict effects relevant to human health, and suggested that effluents of disinfection from different treatments lacked genotoxic compounds capable of causing DNA damage.

4.3 Comprehensive evaluation

Experiments results of different genetic endpoints were summarized in Table 7. Ames and Cho-k1 tests were negative in the dose groups designed for both processes. Mouse bone marrow phagocytic polychromocyte (PCE) and mouse sperm abnormality test displayed positive at high dose 40L/kg·bw of disinfectant water. Comet experiment showed that when water sample was 40L/kg·bw, the filtered water and disinfectant water from the conventional treatment process were positive, while the disinfectant water from recycling process was only positive. The above results indicated that different genotoxicity tests reflect different toxicity results, which may be related to sensitivity and specificity of the tested animals. Although mouse sperm abnormality test and PCE test were positive at 40L/kg·bw, the concentration was 100 times of normal drinking water, which was not consistent with the normal feeding behavior of animals. The same phenomenon also occurred in the high-dose group of comet assay, so it can be considered that effluent from reuse of production wastewater will not cause accumulation of the above genetic toxicity. In addition, it is found that there was no accumulation of organic matter in recycling process when it runs for a long time according to test results of organic matter monitoring in section 3.2. The reuse of wastewater did not increase concentration of contamination inducing the genetic mutation, chromosome damage and germ cell mutation.

Table 7 Results of a series test on different genetic endpoints

Genetic end point	Name of test	Process types	Test results of filtered water	Test results of disinfectant water	Test dose with positive results
Genetic mutations	Ames assay	Conventional process	-	-	
		Recycling Process	-	-	
	CHO-K1	Conventional process	-	-	
	MN test	Recycling Process	-	-	
		Conventional process			4 L/kg·bw
	PCE		-	+	40L/kg·bw□100×□1000×□
Chromosomal aberration	MN test	Conventional process	-	-	
	Sperm abnormality test in mice	Recycling Process	-	+	40L/kg·bw □1000×□
		Conventional process	-	-	
DNA damage	Comet	Conventional process	–	+	40L/kg·bw
	assay	Conventional process	-	+	40L/kg·bw

Note:+ positive results ;- negative results;

Conclusions

In this paper, the genotoxicity of filtered water and disinfectant water from conventional process and reuse process was studied by different genetic end point tests. This work demonstrated more comprehensive assessment of genotoxic effects of reusing residual sludge in drinking water treatment. Results showed a consistent absence of genotoxic and mutagenic effects in all the short-term bioassays performed on bacteria (Ames test) and on mammalian cell (Comet and micronucleus tests) with concentrated effluent derived from different treatments. Generally, there was no genotoxic activity detected at lower concentration ratio (equivalent to the human daily water requirement) in all experiments. Some toxic substances may release after chlorine disinfection, but no relationship was found between the results and somatic variation. Toxicological method was firstly used to evaluate drinking water quality and results indicated that sludge recycling strategy could be feasible for drinking water treatment. Reusing residual sludge had remarkable effect on saving drug dose and increasing drinking water yield, which should be widely recommended.

Declarations

6.1 Ethical Approval and Consent to participate: Not applicable

6.2 Consent for publication

The author confirms:

that the work described has not been published before ;

that it is not under consideration for publication elsewhere;

that its publication has been approved by all co-authors;

6.3 Availability of supporting data

Please see the profile of supplementary

6.3 Competing interests

The authors declare that they have no competing financial interests.

6.4 Funding

This work was supported by Innovation and Enhancing College Project of Guangdong Province, China (2017GkQNCX067, 2017GKTSCX065) and Shenzhen Science and Technology Innovation Committee, China (JCYJ20160226092135176, GJHZ20180416164721073).

6.5 Author’s contributions

Meng and Ting made substantial contributions to conception and design, including acquisition of data, analysis andinterpretation of data;

Meng has draft the article and Zhilin revising it critically for important intellectual content; Qibao and Shaofeng made fnal approval of the version to be published.

6.6 Acknowledgements

This paper would not have been completed without the consistent and valuable reference materials that I received from all co-authors. I would like to express my gratitude.

6.7 Author’s information

Dr. Yao meng (1987-), lecture, full time teacher, in the school of transportation and environment, Shenzhen Institute of Information Technology. She is mainly engaged in water pollution control and related research work. The research focuses on the typical solid-liquid separation in drinking water treatment process and its derived problems.

References

APHA, A., WEF (2012) Standard Methods for the Examination of Water and Wastewater, twenty-second ed. American Public Health Association, Washington, DC.

Arkhipchuk, V.V. and Garanko, N.N. (2005) Using the nucleolar biomarker and the micronucleus test on in vivo fish fin cells. Ecotoxicology and Environmental Safety 62(1), 42-52.

Babatunde, A.O. and Zhao, Y.Q. (2010) Leachability and leaching patterns from aluminium-based water treatment residual used as media in laboratory-scale engineered wetlands. Environmental Science and Pollution Research 17(7), 1314-1322.

Benigni, R. and Bossa, C. (2011) Alternative strategies for carcinogenicity assessment: an efficient and simplified approach based on in vitro mutagenicity and cell transformation assays. Mutagenesis 26(3), 455-460.

Bernardi, M., Adami, V., Albiero, E., Madeo, D., Rodeghiero, F. and Astori, G. (2014) Absence of micronucleus formation in CHO-K1 cells cultivated in platelet lysate enriched medium. Experimental and Toxicologic Pathology 66(2-3), 111-116.

Bertanza, G., Papa, M., Pedrazzani, R., Repice, C., Mazzoleni, G., Steimberg, N., Feretti, D., Ceretti, E. and Zerbini, I. (2013) EDCs, estrogenicity and genotoxicity reduction in a mixed (domestic plus textile) secondary effluent by means of ozonation: A full-scale experience. Science of the Total Environment 458, 160-168.

Chen, T., Xu, Y.P., Liu, Z.Q., Zhu, S.J., Shi, W.X. and Cui, F.Y. (2016) Evaluation of drinking water treatment combined filter backwash water recycling technology based on comet and micronucleus assay. Journal of Environmental Sciences 42, 61-70.

Chen, T., Xu, Y.P., Zhu, S.J. and Cui, F.Y. (2015) Combining physico-chemical analysis with a Daphnia magna bioassay to evaluate a recycling technology for drinking water treatment plant waste residuals. Ecotoxicology and Environmental Safety 122, 368-376.

Crebelli, R., Conti, L., Monarca, S., Feretti, D., Zerbini, I., Zani, C., Veschetti, E., Cutilli, D. and Ottaviani, M. (2005) Genotoxicity of the disinfection by-products resulting from peracetic acid- or hypochlorite-disinfected sewage wastewater. Water Research 39(6), 1105-1113.

Elangovan, N., Chiou, T.J., Tzeng, W.F. and Chu, S.T. (2006) Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. Toxicology 222(1-2), 60-70.

Fenech, M. (2007) Cytokinesis-block micronucleus cytome assay. Nature Protocols 2(5), 1084-1104.

Hallak, J. (2017) Utility of sperm DNA fragmentation testing in different clinical scenarios of male reproductive abnormalities and its influence in natural and assisted reproduction. Translational Andrology and Urology 6, S509-S512.

Ippolito, J.A., Barbarick, K.A., Elliott, H.A (2011) Drinking water treatment residuals: a review of recent uses. Journal of Envrionmental Quality 40, 12.

Jangkorn, S., Kuhakaew, S., Theantanoo, S., Klinla-Or, H. and Sriwiriyarat, T. (2011) Evaluation of reusing alum sludge for the coagulation of industrial wastewater containing mixed anionic surfactants. Journal of Environmental Sciences 23(4), 587-594.

Kravchuk, O., Prodanchuk, M., Nedopytanska, N., Tkachuk, T., Bubalo, V., Tkachuk, O., Zubko, O. and Kostik, O. (2018) Genotoxicity testing of lambda cyhalothrin in fluctuation ames assay and mice bone marrow micronucleus test. Toxicology Letters 295, S155-S155.

Laudat, A. and Lecourbe, K. (2008) Decrease in the percentage of acrosome abnormalities after a migration-survival test of sperm cells. Immuno-Analyse & Biologie Specialisee 23(6), 394-397.

Lin, L., Xu, X.S., Papelis, C. and Xu, P. (2017) Innovative use of drinking water treatment solids for heavy metals removal from desalination concentrate: Synergistic effect of salts and natural organic matter. Chemical Engineering Research & Design 120, 231-239.

Liviac, D., Creus, A. and Marcos, R. (2011) Genotoxic evaluation of the non-halogenated disinfection by-products nitrosodimethylamine and nitrosodiethylamine. Journal of Hazardous Materials 185(2-3), 613-618.

Maffei, F., Carbone, F., Forti, G.C., Buschini, A., Poli, P., Rossi, C., Marabini, L., Radice, S., Chiesara, E. and Hrelia, P. (2009) Drinking water quality: An in vitro approach for the assessment of cytotoxic and genotoxic load in water sampled along distribution system. Environment International 35(7), 1053-1061.

Makris, K.C., Harris, W.G., O'Conno, G.A. and Obreza, T.A. (2004) Phosphorus Immobilization in Micropores of Drinking-Water Treatment Residuals: Implications for Long-Term Stability. Environmental Science & Technology 38(24), 6590-6596.

Misik, M., Knasmueller, S., Ferk, F., Cichna-Markl, M., Grummt, T., Schaar, H. and Kreuzinger, N. (2011) Impact of ozonation on the genotoxic activity of tertiary treated municipal wastewater. Water Research 45(12), 3681-3691.

Moghaddam SS, M.M., Arami M (2011) Response surface optimization of acid red 119 dye from simulated wastewa ter using Al based waterworks sludge and polyaluminium chloride as coagulant. Journal of Environmental Managment 92(4), 1284.

Nair, A.T. and Ahammed, M.M. (2015) The reuse of water treatment sludge as a coagulant for post-treatment of UASB reactor treating urban wastewater. Journal of Cleaner Production 96, 272-281.

Peycheva, E., Alexandrova, R. and Miloshev, G. (2014) Application of the yeast comet assay in testing of food additives for genotoxicity. Lwt-Food Science and Technology 59(1), 510-517.

Punamiya, P., Sarkar, D., Rakshit, S. and Datta, R. (2013) Effectiveness of Aluminum-based Drinking Water Treatment Residuals as a Novel Sorbent to Remove Tetracyclines from Aqueous Medium. Journal of Environmental Quality 42(5), 1449-1459.

Qi, L., Liang, H., Wang, Y. and Li, G.-b. (2009) Integration of immersed membrane ultrafiltration with the reuse of PAC and alum sludge (RPAS) process for drinking water treatment. Desalination 249(1), 440-444.

Ragazzo, P., Feretti, D., Monarca, S., Dominici, L., Ceretti, E., Viola, G., Piccolo, V., Chiucchini, N. and Villarini, M. (2017) Evaluation of cytotoxicity, genotoxicity, and apoptosis of wastewater before and after disinfection with performic acid. Water Research 116, 44-52.

Sadri Moghaddam, S., Alavi Moghaddam, M.R. and Arami, M. (2010) Coagulation/flocculation process for dye removal using sludge from water treatment plant: Optimization through response surface methodology. Journal of Hazardous Materials 175(1), 651-657.

Shi, Y., Cao, X.W., Tang, F., Du, H.R., Wang, Y.Z., Qiu, X.Q., Yu, H.P. and Lu, B. (2009) In vitro toxicity of surface water disinfected by different sequential treatments. Water Research 43(1), 218-228.

Singireesu, S.S.N.R., Misra, S., Mondal, S.K., Yerramsetty, S., Sahu, N. and Babu, K.S. (2018) Costunolide induces micronuclei formation, chromosomal aberrations, cytostasis, and mitochondrial-mediated apoptosis in Chinese hamster ovary cells. Cell Biology and Toxicology 34(2), 125-142.

Tabrez, S., Shakil, S., Urooj, M., Damanhouri, G.A., Abuzenadah, A.M. and Ahmad, M. (2011) Genotoxicity Testing and Biomarker Studies on Surface Waters: An Overview of the Techniques and Their Efficacies. Journal of Environmental Science and Health Part C-Environmental Carcinogenesis & Ecotoxicology Reviews 29(3), 250-275.

Vasiljevic, J., Zivkovic, L., Cabarkapa, A., Bajic, V., Djelic, N. and Spremo-Potparevic, B. (2016) Cordyceps sinensis: Genotoxic Potential in Human Peripheral Blood Cells and Antigenotoxic Properties Against Hydrogen Peroxide by Comet Assay. Alternative Therapies in Health and Medicine 22, 24-31.

Visa, M., Isac, L. and Duta, A. (2012) Fly ash adsorbents for multi-cation wastewater treatment. Applied Surface Science 258(17), 6345-6352.

Wang, C.H., Liu, J.F., Wang, Z.X. and Pei, Y.S. (2014a) Nitrification in lake sediment with addition of drinking water treatment residuals. Water Research 56, 234-245.

Wang, C.H. and Pei, Y.S. (2012) The removal of hydrogen sulfide in solution by ferric and alum water treatment residuals. Chemosphere 88(10), 1178-1183.

Wang, C.H., Yuan, N.N. and Pei, Y.S. (2014b) An anaerobic incubation study of metal lability in drinking water treatment residue with implications for practical reuse. Journal of Hazardous Materials 274, 342-348.

Wang, C.H., Yuan, N.N. and Pei, Y.S. (2014c) Effect of pH on Metal Lability in Drinking Water Treatment Residuals. Journal of Environmental Quality 43(1), 389-397.

Wang, M.C., Hull, J.Q., Joa, M., Dempsey, B.A., Cornwell, D.A (1992) Engineering behaviour of water treatment sludge. Jouranl of Envrionmental Engineering 118, 6.

Xu, Y., Chen, T., Cui, F. and Shi, W. (2016a) Effect of reused alum-humic-flocs on coagulation performance and floc characteristics formed by aluminum salt coagulants in humic-acid water. Chemical Engineering Journal 287, 225-232.

Xu, Y.P., Chen, T., Liu, Z.Q., Zhu, S.J., Cui, F.Y. and Shi, W.X. (2016b) The impact of recycling alum-humic-floc (AHF) on the removal of natural organic materials (NOM): Behavior of coagulation and adsorption. Chemical Engineering Journal 284, 1049-1057.

Yuan, N.N., Wang, C.H. and Pei, Y.S. (2016a) Investigation on the eco-toxicity of lake sediments with the addition of drinking water treatment residuals. Journal of Environmental Sciences 46, 5-15.

Yuan, N.N., Wang, C.H., Pei, Y.S. and Jiang, H.L. (2016b) Applicability of drinking water treatment residue for lake restoration in relation to metal/metalloid risk assessment. Scientific Reports 6.

Zhong, Y., Feng, S.L., Luo, Y., Zhang, G.D. and Kong, Z.M. (2001) Evaluating the genotoxicity of surface water of Yangzhong City using the Vicia faba micronucleus test and the comet assay. Bulletin of Environmental Contamination and Toxicology 67(2), 217-224.

Figures

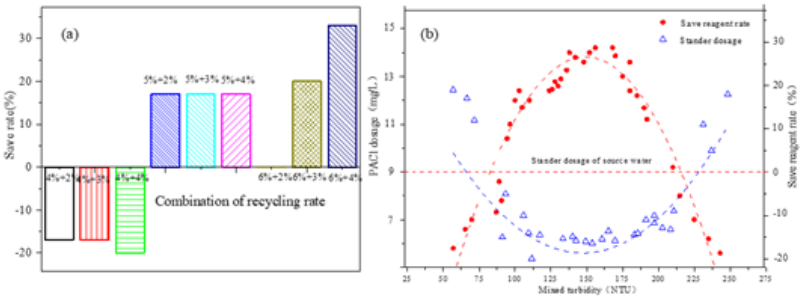

Figure 1

The optimal recycling conditions with mixed wastewater: (a) combination of recycling rate, (b)mixed wastewater turbidity

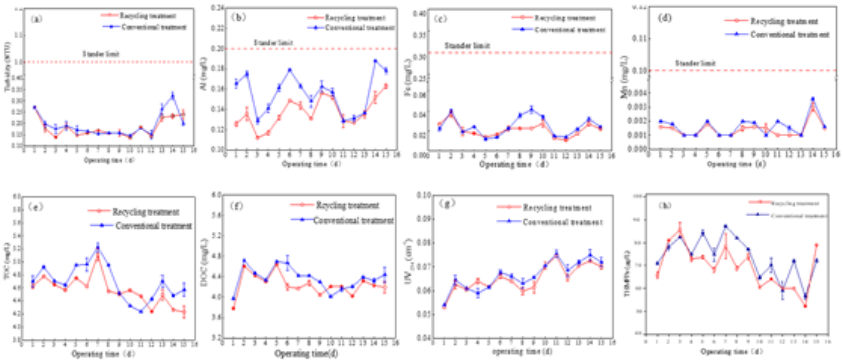

Figure 2

Influence of continuous recycling sludge and filter backwash water for 15 days on: a)Turbidity, b)Al, c)Fe, d)Mn, e) TOC, f)DOC, g)UV254, h) THMFPs

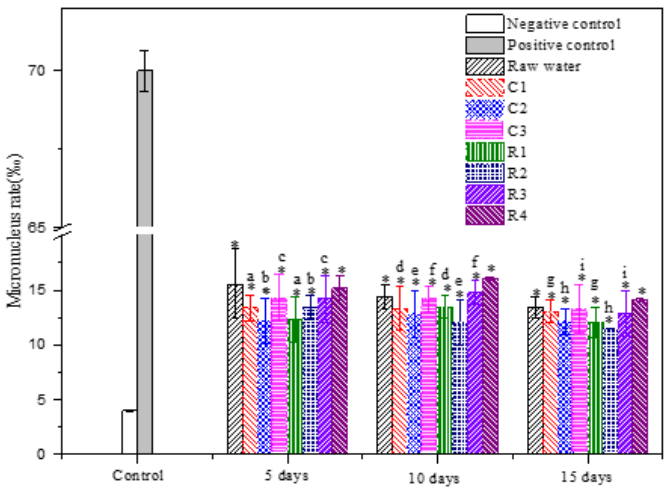

Figure 3

Micronucleus (MN) assay on zebra fish of drinking water sampled at different points of plants between conventional and recycling process

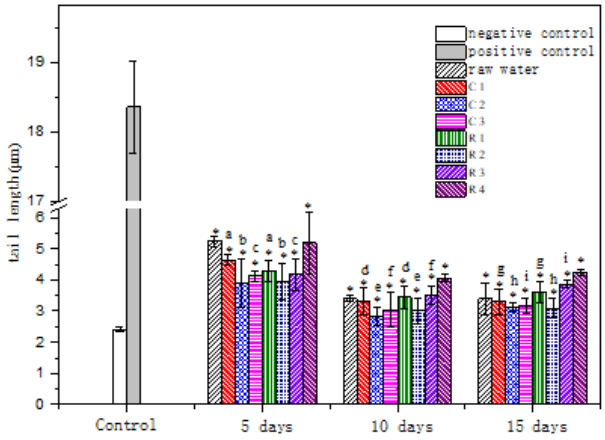

Figure 4

Comet assay on zebra fish of drinking water sampled at different points of plants between conventional and recycling process （note：*=p＜0.05，there was a significant difference from the blank test；the letter（a，i）=p＞0.05，there is no significant difference in water samples of the sampling points corresponding to each processing unit of the two processes)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementary.doc
- Formula1.jpg