Journal of Cleaner Production 185 (2018) 523–532

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Biological damage to Sprague-Dawley rats by excessive anions contaminated groundwater from rare earth metals tailings pond seepage

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ARTICLE INFO

Article history:
Received 17 July 2017
Received in revised form
6 March 2018
Accepted 7 March 2018
Available online 9 March 2018

Keywords:
DNA damage
Kidney
Fluoride
Chloride
Biosafety evaluation

ABSTRACT

Current research on groundwater contamination due to mining tailing ponds seepage has concentrated on the toxicological effects of metals on organisms and ecosystems. However, recent studies found that the most hazardous pollutants that affect water quality in some rare earth metals smelting tailings pond are the large amount of ions but not metals. Whether these excess ions can cause genetic damage in organisms needs further study. Thus, the hazardous contaminant components in groundwater from five different sites in and near a rare earth elements (lanthanides) tailings pond were analyzed. Then, the biological damages to Sprague-Dawley (SD) rats caused by tailings seepage-contaminated groundwater at the individual, organ, tissue, and cellular levels were systematically studied. Following that, the correlations between the pollution components in the contaminated groundwater and tissue damages in SD rats were further analyzed. The results showed that the main hazardous pollution ions in the rare earth metals tailings seepage-contaminated water were F^-, Cl^-, and SO_4^{2-}. Contamination was increasingly severe closer to the tailings dam. Water from the study sampling sites caused liver and kidney damage to the SD rats. Further, the results from microscopic morphology and flow cytometric apoptosis analyses showed that the damages caused to the kidney epithelia cells by F^- and Cl^- showed an increasing trend as the sites neared the tailings dam, and there were positive correlations. The effect of SO_4^{2-} was not significant. Therefore, this study provides a foundation to scientifically and effectively evaluate the genetic damages caused to organisms by groundwater near tailings ponds, and also provides a theoretical basis to reveal the mechanism underlying this effect.

1. Introduction

Rare earths(lanthanides) mining exploitation has brought enormous economic benefits to mankind, but a tremendous impact on the ecological environment in and around the mining area (Migaszewski and Galuszka, 2015; Wang et al., 2017). Rare earth metals tailing ponds are used to store waste generated during processes such as beneficiation and coking. In the early times, ecological security was not considered for site selection of tailing ponds. As a result, a large amount of wastewater from beneficiation was added to the ponds, causing the pollutant concentrations to increase year after year (Huang et al., 2016). At present, the pollutants have continued to leak into the ground, contaminating the local groundwater, and causing considerable concern for national and international scholars (von der Heyden and New, 2004; Allison et al., 2015; Hao et al., 2016; Tiwar and Maio, 2017). It was reported that seepage from these tailings ponds not only caused pollution of the surrounding groundwater, but also led to multiple problems,

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https://doi.org/10.1016/j.jclepro.2018.03.074
0959-6526/

such as soil salinization, surface water pollution, ecological degradation, and even affected human health (Liang et al., 2014; Tiwari et al., 2015; Romero-Freire et al., 2016).

A rare earths(lanthanides) tailing pond is located in southwest of china's Baotou, which covers an area of 11 square kilometers and 15 million cubic meters of reservoir water. There are around many of iron ore dressing plants, the microcrystalline and coal chemical plants. Therefore, the wastewater from these ores dressing is discharged into the pond, and has brought about many pollution problems. Local environmental monitoring stations examining the groundwater leakage surrounding the pond showed that this groundwater exhibited varying degrees of pollution and contaminated relatively lower concentrations of metals and higher concentration of ions. This result was also confirmed by Miao (2013) and other scholars (Si et al., 2016). It is noteworthy that studies have shown that groundwater contaminated with seepage from this tailings pond could cause a certain degree of biological damages. Zornoza and An reported that groundwater with rare earth metasl tailings pond seepage would change the local microbial ecological structure (Zornoza et al., 2015; An et al., 2016); In addition, Thomas (2014) directly demonstrate that rare earths elements (lanthanides) would inhibit plant germination. However, these studies were limited to the investigation and research on the ecological environment and plants surrounding the tailings ponds, and few studies have determined whether it could cause genetic damage in animals and mankind. Therefore, it is necessary to identify the extent of water pollution and the hazardous components of contamination in areas surrounding tailings ponds, to further define the degree of local genetic damage in animals and mankind caused by groundwater contaminated from tailings pond seepage, and to conduct an in-depth study on the interaction mechanism of pollution components in water surrounding tailings ponds and genetic damages. These efforts will have great significance for the scientific and effective assessment of the risk of groundwater contaminated with tailings pond seepage.

Based on this, taking a rare earths(lanthanides) tailing pond in northern China as the research object, SD rats used as the experimental material were selected. This is due to its high homology with humans, and this animal is widely used in the field of pharmacology, oncology, developmental biology, and neurobiology. It is an extremely effective experimental material to assess the health of human disease (Guo et al., 2015; Yonezawa et al., 2015). Therefore, the quality and content of the hazardous pollution components of the groundwater surrounding the pond were analyzed. Then, this was followed by a systematic assessment of the water quality of the environment surrounding the tailings pond to determine the extent of biological damage caused to the SD rats at the individual, tissue, and cellular levels, and to further confirm the relationship between pollution components and the genetic damage in the SD rats.

2. Material and methods

2.1. Materials

The SD rats were provided by the Experimental Animal Center of Inner Mongolia University, and the source of animals met the ethics requirements.

2.2. Methods

2.2.1. Collection of water samples and measurement of water quality parameters

In this study, the water sampling points were set in the area surrounding a rare earth metals tailing pond in northern China, and five sites were selected at various distances to the west of the pond (a residential area is located to the west, and rare earths(lanthanides) industrial areas are located in the other directions, Fig. 1). These sites (ranked from near to far from the pond) were as follows: tailings pond water (sample 1, S1), seepage water outside the tailings pond (sample 2, S2), Dalahai up-village shallow well water (sample 3, S3), Dalahai down-village shallow well water (sample 4, S4), and the city's drinking water as the standard control group (sample 5, S5). At each sampling period, a 20 μL water sample was collected for direct measurements of ion contents using the equipment (MIC-II ion chromatograph, Met Rohm), and the metals were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Icap6000, Thermo Scientific) according to Thomas (2004). Moreover, ICP-MS was calibrated daily with a certified standard solution (Merck ICP Multi-elements standard solution XIII), and the R^2 value of the calibration curve was >0.999 for each metals. Using a control solution, the calibration was checked after every 10 samples. If the deviation was >10%, the device was recalibrated. The experiment was preformed three times.

2.2.2. Examination of groundwater surrounding the tailings pond and DNA damage in SD rats

2.2.2.1. Detection at the individual level of SD rats. Healthy adolescent female SD rats (40 ± 2 d old), with an average weight of 28.5 g, pre-feed for 3 days, were chosen for the experiment. According to a randomized complete block design, the rats were divided into five groups, consisting of five rats in each group. At this time, the food and growth environment were the same for each group. In addition, the five treatments consisted of S1, S2, S3, S4, and S5 (the control). SD rats were fed with the water samples once every 4 h, 0.5 mL each time by oral gavage; the feeding cycle lasted one month. The body weight and growth conditions of the SD rats were recorded every day to determine the impact of the water surrounding the tailings pond on the growth and development of individual rats.

2.2.2.2. Detection at the organ level of SD rats. The degree of tissue damage was detected by organ/body weight ratio (Zhang et al., 2016). Briefly, after the female SD rats were treated with the 5 samples described above for 30 days, 3 rats were randomly chosen from each group, and their main organs (heart, liver, lung, spleen, kidney) and their bodies were harvested and washed with physiological saline, weighed after drying with filter paper, and the ratio of the organ weight to body weight were calculated to evaluate the relationship between polluted groundwater and the biological response of tested organs. Therefore, the damage impact were statistically analyzed from the water surrounding the tailings pond at the tissue level.

2.2.2.3. Detection at the tissue level of SD rats. The hematoxylin and eosin (H&E) staining was used as histopathology analysis. Specifically, tissue samples were derived from the above-mentioned experiments with significant damage to the organs, fixed tissues with 10% formalin for 24 h at room temperature. Trimmed fixed tissues into appropriate size and shaped and placed in embedding cassettes. Processed for paraffin embedding schedule as follow: 70% Ethanol, two changes; 80% Ethanol, 1 change; 95% Ethanol, 1 change; 100% Ethanol, 3 changes; Xylene, 3 changes; Paraffine wax (58–60 °C), 2 changes, Embedding tissues into paraffin blocks. Trimmed paraffin blocks as necessary and cut at 5 μm. Placed paraffin ribbon in water bath at about 40–45 °C. Mountted sections onto slides. Allowed sections to air dry for 30 min and then baked in 45–50 °C ovens overnight. The deparaffinize sections in 2–3 changes of xylene, 10 min each. Hydrate in 2 changes of 100%