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Spatio-temporal distribution of perchlorate and its toxicity in *Hydrilla verticillata*

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ABSTRACT

The spatio-temporal distribution of perchlorate in water sources around an ammonium perchlorate production unit and its toxicity response on a dominant aquatic plant, *Hydrilla verticillata* are reported in this study. Samples (n = 453) from ground water (open well) and surface water sources within 5 km from the production unit over a period of 12 months (2014, June – 2015, May) were screened for ClO_4^-. During the period, ClO_4^- concentration in ground water samples close to the production unit increased to > 40,000 µg/L, and ClO_4^- was detected at 1740 µg/L in well water 1.6 km away from the production unit. A community pond in the area also showed an increase in ClO_4^- level up to 29,000 µg/L. In all water sources, ClO_4^- level was maximum during the rainy season (July, monsoon). A natural degradation of ClO_4^- was not observed in the area as evident from its persistent level and spreading to more areas. *H. verticillata*, the dominant vegetation in the pond exhibited severe toxic response like massive decay and loss of photosynthetic pigments such as chlorophyll and carotene due to ClO_4^- exposure. The plant accumulated ClO_4^- up to 60 ± 0.8 mg/kg wet weight with a Bio-Concentration Factor 2.06 ± 0.005. This is the first report on spatio-temporal distribution of ClO_4^- at higher levels in a natural environment and its toxicity response to plants under natural condition.

1. Introduction

Environmental contamination of ClO_4^- is becoming a serious public health concern due to its adverse effect on thyroid gland functioning, and widespread distribution of ClO_4^- in various water sources and foodstuffs (Calderon et al., 2017; Charnley, 2008; Isobe et al., 2013; Srinivasan and Sorial, 2009). The current health advisory level for ClO_4^-, based on the reference dose recommended by National Academy of Sciences is 15 µg/L (US EPA, 2008). The World Health Organization (WHO) established provisional maximum tolerable daily intake (PMTDI) of 0.01 mg/kg body weight for ClO_4^- (WHO, 2010).

The release of ClO_4^- to the environment is primarily associated with its extensive use in aerospace programs, defense R & D activities/operations and a few industries like fireworks manufacturing, bleaching, tanning etc. (ITRC, 2005; Trumpolt et al., 2005; Voogt and Jackson, 2010). Published reports on ClO_4^- contamination on the surface as well as groundwater sources in various countries are summarized in a table in the supplementary materials (Table S1). Perchlorate is highly soluble in water, resistant to chemical and biological degradation under natural conditions and it does not adsorb to natural mineral surfaces or organics (Stetson et al., 2006; Urbansky, 2002). However, the transport of ClO_4^- in groundwater is extremely slow, probably due to ClO_4^- salting out at high concentrations leading to its slow migration relative to groundwater velocity (Brown and Gu, 2006). To the best of our knowledge, the only reported study on the spatio-temporal distribution of ClO_4^- in water was in Potomac River in the US, where ClO_4^- concentration ranged from 0.03 µg/L to 7.63 µg/L (mean 0.67 ± 0.97 µg/L) during one year period (Impellitteri et al., 2011). The maximum ClO_4^- concentration reported so far was in the ground water, contaminated with discharge from firework manufacturing industrial area in India (Isobe et al., 2013). However long-term monitoring data of ClO_4^- contamination in an area and associated toxicity response is lacking.

The toxicity response of plants and animals exposed to ClO_4^- was reported in a few previous studies, mostly under controlled laboratory conditions. In higher animals (including human), perchlorate ingestion

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causes inhibition of iodide transportation to thyroid gland follicle cells leading to hypothyroidism (Greer et al., 2002; Ting et al., 2006). The decrease in serum thyroid hormones (T3 and T4), can lead to neuro-development disorders and increase in serum TSH level leading to thyroid hyperplasia and tumors (Wolff, 1998). In addition to interfering with thyroid gland function, experimental studies with animals have also revealed alterations in phenotype, tumor development and changes in the reproductive system due to ClO_4^- exposure (Mattie et al., 2006; Mukhi and Patiño, 2007; Petersen et al., 2016).

Phytotoxic studies with plants showed uptake, translocation and accumulation of ClO_4^- in both aquatic and terrestrial plants (Sundberg et al., 2003; Tan et al., 2004; Xie et al., 2009). The toxic response of ClO_4^- on plants mainly included growth inhibition, loss of photosynthetic pigments as well as alterations in physiological enzyme activities. He et al., have reported variation in physiological characteristics and chlorophyll content in four different wetland plants exposed to ClO_4^- at 20 − 500 mg/L levels (He et al., 2013). It was evident from previous studies that phytotoxic response of ClO_4^- depends mainly on factors like plant species, ClO_4^- concentration, and exposure duration (He et al., 2013; Tan et al., 2004; Xie et al., 2014). It was also speculated that the toxic response of ClO_4^- on plants is mainly due to its high oxidising property. Understanding the spatio-temporal distribution of ClO_4^- in a contaminated area as well as assessing its toxic response is highly important to assess its environmental impact and to formulate strategies for managing its contamination. But, studies in this area is very limited and confined to lab studies under controlled conditions. Therefore, the present study aims to analyse the spatio-temporal distribution of ClO_4^- in a severely contaminated area and to monitor the toxicity response of ClO_4^- on the dominant aquatic plant in a contaminated natural pond.

2. Materials and methods

2.1. Study area and sample type

This study was conducted in an area covering 5 km around an ammonium ClO_4^- production unit in Aluva, Ernakulam district, Kerala (India). The sampling site was selected based on our previous study which detected a high level of ClO_4^- in drinking water (Nadaraja et al., 2015). A comprehensive sampling map showing ground water and surface water sampling points and location of a production unit are presented in Fig. 1.

In the present study, an extensive analysis of ClO_4^- in water samples from open wells (labelled as G) and three surface water sources (pond, streams and a river) (labelled as S) was carried out over a period of one year (June 2014 − May 2015). Water samples (n = 341) collected from household open wells in the area was the source of the ground water sample. Special attention was paid to three community wells - W1 (G92), W2 (G93) and W3 (G94); average ~10 m depth in the close proximity of the production unit (~100–300 m away). Samples were collected every month from these wells, which were the main source of drinking water for the residents in the area, comprising of 180 families (~450 people). Samples (n = 72) were also collected from five different sites (S1 − S5) in a community pond covering 640 m^2 area, average depth 5 m, located ~600 m from the production unit. In addition to ClO_4^-, other water quality parameters such as pH, NO_3^-, Cl^-, PO_4^-, SO_4^{2-} and NH_3^+ in the pond samples were also monitored. A schematic of the pond, its location and sample points are presented in supplementary material (Fig S1). The pond water was used by the residents for feeding cattle, washing and other domestic purposes. A narrow canal (~2 feet wide, ~2 feet depth) was found flowing into the pond close to site S4, and water samples (S11 and S12) (n = 12) from this canal was analysed for ClO_4^-, but the origin of this canal could not be traced. We also observed a small stream (S0) running out of the pond and ClO_4^- was analysed in samples (n = 12) from this stream. Water sample (n = 16) from five different points (S6–S10) from Periyar River, a major source of drinking water supply located 3 − 5 km from the production unit, was also collected for ClO_4^- analysis. A total number of 453 water samples from various sources were collected for ClO_4^- analysis during the study period.

2.2. Analysis of perchlorate in soil samples

Soil samples collected from different points in the premises of production unit were tested for ClO_4^-. One gram of soil from each sampling point was suspended in 50 ml distilled water taken in a 250 ml conical flask and mixed vigorously by keeping in a shaker at 250 rpm for 10 min. The suspension was then filtered and the clear supernatant was collected. The steps were repeated twice to ensure complete extraction of ClO_4^-. The extracts were pooled together for ClO_4^- estimation was expressed as mg/g soil (wet weight).

2.3. Contour mapping of the study area

A contour mapping was done to assess the spatial distribution of ClO_4^- during the period of study. The surface was created from the sample points using GIS techniques. ClO_4^- value of well water samples (n = 74) during June 2014 and May 2015 were selected for two separate plots, based on that extent of contamination was assessed. The points of ClO_4^- analysis data and latitude and longitude values were interpolated using IDW (Inverse distance weighted) technique of spatial analyst extension of ARC Map 10.2.2.

2.4. Toxic response of perchlorate to aquatic vegetation

The toxic response of ClO_4^- to the dominant aquatic plant (test) in the contaminated pond was studied. The taxonomic identification of the plant was done with the help of a taxonomic expert using suitable identification keys.

The plants were collected, brought to the laboratory and cleaned by flushing with distilled water prior to further analysis. Perchlorate induced toxicity on the plant was investigated in terms of physical appearance and photosynthetic pigment content. The observation was compared with similar plant collected from an uncontaminated pond (control).

2.4.1. Morphological variations

Morphological features like leaf area, the number of leaves per node, leaf colour and thickness, stem colour, stem thickness and intermodal distance were recorded for both sets of plant samples. A minimum of three randomly selected plant samples was analysed from both the sets.

2.4.2. Photosynthetic pigment (Chlorophyll and Carotenoids) content

The effect of ClO_4^- on the level of photosynthetic pigments, chlorophyll and carotenoids in both test and control plants were studied. To detect variations in chlorophyll content, leaves from both plants were taken for direct observation under a fluorescence microscope (Leica, DM 2500) at 680 nm. The presence or absence of red fluorescence due to auto-fluorescence of chlorophyll was recorded and images were taken. The quantification of chlorophyll (Chl_a and Chl_b) and total carotenoids (T_{car}) of both sets of plant leaves were done by acetone incubation method (Lichtenthaler and Buschmann, 2001). Plant material (1 g) was crushed and added to a 50 ml glass tube containing 25 ml buffered acetone and incubated in a dark place with occasional shaking to extract pigments. The extract was then filtered and absorbance was read at 470, 645, 663 and 710 nm using a UV − VIS spectrophotometer (Thermo Fisher) for measuring Chl_a, Chl_b and T_{car} respectively. The total chlorophyll was calculated from Chl_a and Chl_b values.