

Original article

Bacterial community structures as a diagnostic tool for watershed quality assessment

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Abstract

The analysis of bacterial community structures can be considered a promising instrument when assessing the quality and health of a body of water. Here, the representation of a new biological approach to studying such pollutant-based impact on freshwater sediments is explored. To test our hypothesis, sediment samples of *Phragmites australis* (common reed)-associated rhizosphere were collected at sites affected by different types and levels of pollution, all located in Zhangye, Gansu Province, China. The analyzed bacterial community structures showed a varying pattern according to the presence, characteristics and level of contaminants. Results of the study showed that bacterial community structures could be effectively used as diagnostic tools to map watershed quality status.

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Keywords: Bacterial community structure; Bioindicator; Freshwater; *Phragmites australis*; Environmental pollution

1. Introduction

Bioindicators are organisms that rapidly reveal the quality of an environment, by highlighting the effect of environmental changes on a population, community, habitat or even on an ecosystem [1]. On the basis of this characterization, bioindicators are useful in assessing additive, synergistic and antagonistic effects upon living organisms within a broad pollutant-based spectrum of complex environments [1,2]. In the last few decades, several bioindicators for freshwater environments have been thoroughly studied. Most of these organisms are macrobenthic invertebrates, algae and microorganisms [3–5]. Depending on the type of organism chosen, there exist a variety of scales that can be examined by biomonitoring: individual, multi-population and community-related [1,6,7]. For example, aquatic macroinvertebrates can be considered sensitive sentinels in polluted freshwater; analyses show they can be recorded at different ecological levels.

The response at the individual level is usually linked to a defined type of pollution stress. In contrast, a multi-population examination is slightly more difficult to understand, since it requires more exhaustive testing due to the complexity of additional sources of disturbance [8].

Because of their attributes, bacteria can be considered good potential bioindicators at different organizational levels. Bacteria are ubiquitous and can be assayed at low cost; in addition, they have the highest surface-area-to-volume ratio compared to other organisms. Furthermore, due to the high number of individuals at each microsite, analysis of bacterial communities in terms of an increase or decrease in taxon provides robust results. As a result, the assessment level of pollution within an environmental sample is commonly achieved by analyzing single bacterial species or a limited bacterial population. For example, it is well known that the metabolic pathway of *Vibrio fischeri*'s bioluminescence is useful in detecting xenobiotic pollutants, while fecal bacteria (coliforms and enterococci) are regularly used to measure fecal pollution levels [9,5]. In contrast, use of an entire bacterial community as a bioindicator in freshwater environments has been limited

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to very few published case studies, despite all the attributes that make microbial communities a promising tool for detecting pollutants [10–12]. The aim of this work was to determine whether the structure and diversity of bacterial communities can be useful as a diagnostic tool in monitoring the ecological state of a watershed affected by different levels of pollution. In particular, it takes into consideration the influence of metals and nutrients on a spatial scale. The studied compounds have significant persistence in the environment and a dramatic effect on biota, reflecting anthropogenic input from industrial, agricultural and municipal activities.

2. Materials and methods

2.1. Experimental design and sampling procedures

The study was conducted in the city of Zhangye, the Gansu Province of northern China in the spring of 2012. Zhangye, with a population of 1,200,000 inhabitants, is located in a large oasis surrounded by the Gobi Desert and the Qilianshan Mountains, and the Heihe River is its water source. The city is characterized by a complex network of canals mostly covered by *Phragmites australis* (Cav.) Trin (common reed). Water from the canals predominantly runs into the Heihe River (Fig. 1) [13].

Sampling sites were chosen to provide broad geographic coverage, representing canals suspected to be contaminated by different types and levels of pollution. The area was divided into four different zones according to anthropogenic influences (Fig. 1; Table 1): six sampling points were assessed along the main canal crossings through the main urban area (ZY36–41); four sites were near coal and metallurgy industries in a smaller secondary canal (ZY24 and ZY25) and, again, in the main canal collecting water from the industrial area (ZY22 and

ZY23). Two sites were sampled in Zhangye Natural Park (ZY34 and ZY35), an extensive area completely covered by reeds. Five sites were chosen in the agricultural area: two were collected in an agricultural drainage canal near the Heihe River (ZY29 and ZY30), two on the banks of Heihe (ZY30 and ZY31) and one was between the industrial area and the main city center (ZY21). Finally, an unpolluted site with a low anthropic influence located in the Hatengtaohai area of the Inner Mongolia autonomous region was identified as an external control. The site was chosen because of the presence of *P. australis*, similar environmental conditions and geological composition. However, it originates from a different hydrogeographic basin.

Bacterial communities inhabiting the rhizosphere were sampled due to their increased stability and limited long-distance dispersal with respect to habitats such as bulk sediments via the water column [14,15].

In each of the 18 sampling sites, one rhizosphere sample from three different roots, dispersed in water, was collected, with an average of 10 cm in distance. The rhizosphere was considered to be particles tightly adhering to the root (within 1–3 mm) after vigorous shaking. About 10 g of wet sediment were put into sterile tubes at 4 °C for molecular analysis. Moreover, at each sampling site, sediment associated with *P. australis* was collected in sterile bags to allow for chemical analysis and placed at –20 °C. Tubes were immediately transported to nearby laboratories for rapid DNA extraction and bags were shipped by refrigerated delivery to Italy.

2.2. Chemical and physical analyses of sediments

About 200 g of sediment samples were oven-dried at 105 °C until constant weight and then acid-digested (HNO₃ concentrated 65% and H₂O₂ 30%) in a Milestone high

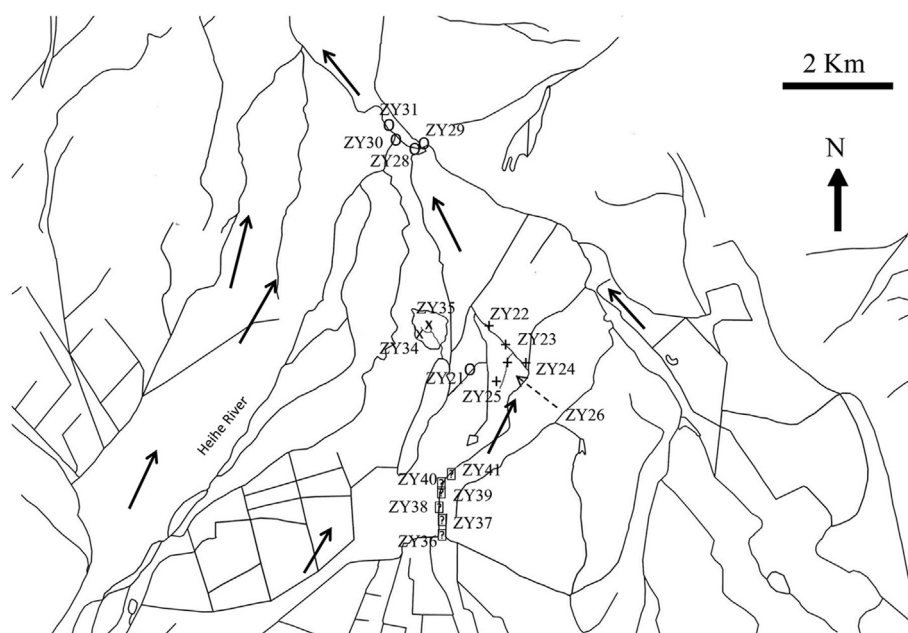


Fig. 1. Map of Zhangye representing the canal system and sampling points: industrial area (+), urban area (□), agricultural area (○) and natural park (×). Arrows represent water efflux directions (modified from Zhang et al., 2005).

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