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Longitudinal survey of lead, cadmium, and copper in seagrass *Syringodium filiforme* from a former bombing range (Vieques, Puerto Rico)

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ABSTRACT

Trace element composition in plant biomass could be used as an indicator of environmental stress, management practices and restoration success. A longitudinal study was conducted to compare Pb, Cd, and Cu content in seagrass *Syringodium filiforme* collected at a former bombing range in Puerto Rico with those of a Biosphere Reserve under similar geoclimatic conditions. Trace elements were measured by atomic absorption after dry-ashing of samples and extraction with acid. In general, levels of Pb, Cd, and Cu varied during 2001, 2003, 2005–2006, and 2013–2016. Results showed that bioaccumulated concentration of these trace elements were consistently higher, but not significant, at the bombing range site. As expected in polluted areas, greater variability in Pb and Cd content were observed in the military impacted site with levels up to 14 and 17 times higher than seagrass from the reference site, respectively. Although a decrease in Pb was observed after cessation of all military activities in 2003, the concentration in plant biomass was still above levels of ecological concern, indicating that natural attenuation is insufficient for cleanup of the site.

1. Introduction

Military practices have left a legacy of pollution worldwide, representing a significant anthropogenic disturbance. In Puerto Rico, the Eastern island municipality of Vieques was used for military practices by the US Navy from mid-1940 until 2003. During the intensive training activities at the 23,000-acre site known as the Atlantic Fleet Weapons Training Facility (AFWTF), significant amounts of live ammunition was fired, including both conventional and unconventional weapons, napalm, agent orange and bullets with depleted uranium. Leaving the population with areas highly contaminated and with the potential risk of developing conditions associated with munitions-specific carcinogens [1]. After cessation of all military activities was ordered, the site was considered as a highest priority for cleanup, including large portions of surrounding waters. Therefore, the area was included in 2004 in the National Priorities List by the US Environmental Protection Agency.

In addition to explosive compounds, military activities have been linked to heavy metal pollution including lead (Pb), cadmium (Cd) and copper (Cu), which are known to be toxic elements [2–6]. Beyond direct exposure and toxic consequences, these elements bioaccumulate in living organisms, including humans and plants. Many preventive measures to decrease the exposure to Pb have been implemented around the world. However, Pb toxicity is still prevalent. Main sources of Pb exposure include mining, smelting, lead battery disposal, crystal and ceramic industries. Pb toxicityinduces a wide array of physiological, biochemical and behavioral dysfunctions. These effects are found in laboratory animals, as well as humans, and affect the nervous, hematopoietic, cardiovascular, and reproductive systems [7].

Cd toxicity has been studied thoroughly. These studies show that, in humans, kidneys are the most affected organs. Other affected organs in animals and humans include liver, lungs, pancreas, bones, reproductive organs, hematopoietic, nervous and cardiovascular systems [8]. Other health conditions attributed to Cd toxicity include hypertension, type 2 diabetes mellitus, thyroid function, and cancer [8,9]. Although Cu is an essential microelement for plants and human bodies, in high concentrations it is toxic to the ecosystem and human health. A constant intake of Cu over an extended period can cause anemia, damage of the pancreas, liver, and kidney, and decrease in the levels of high-density lipoprotein cholesterol [10].

Aquatic ecosystems are more sensitive to heavy metals than terrestrial ecosystems. Specifically, seagrass can bioaccumulate essential and non essential elements from the water column or sediment material, thus compromising the marine food web. The ability of seagrass

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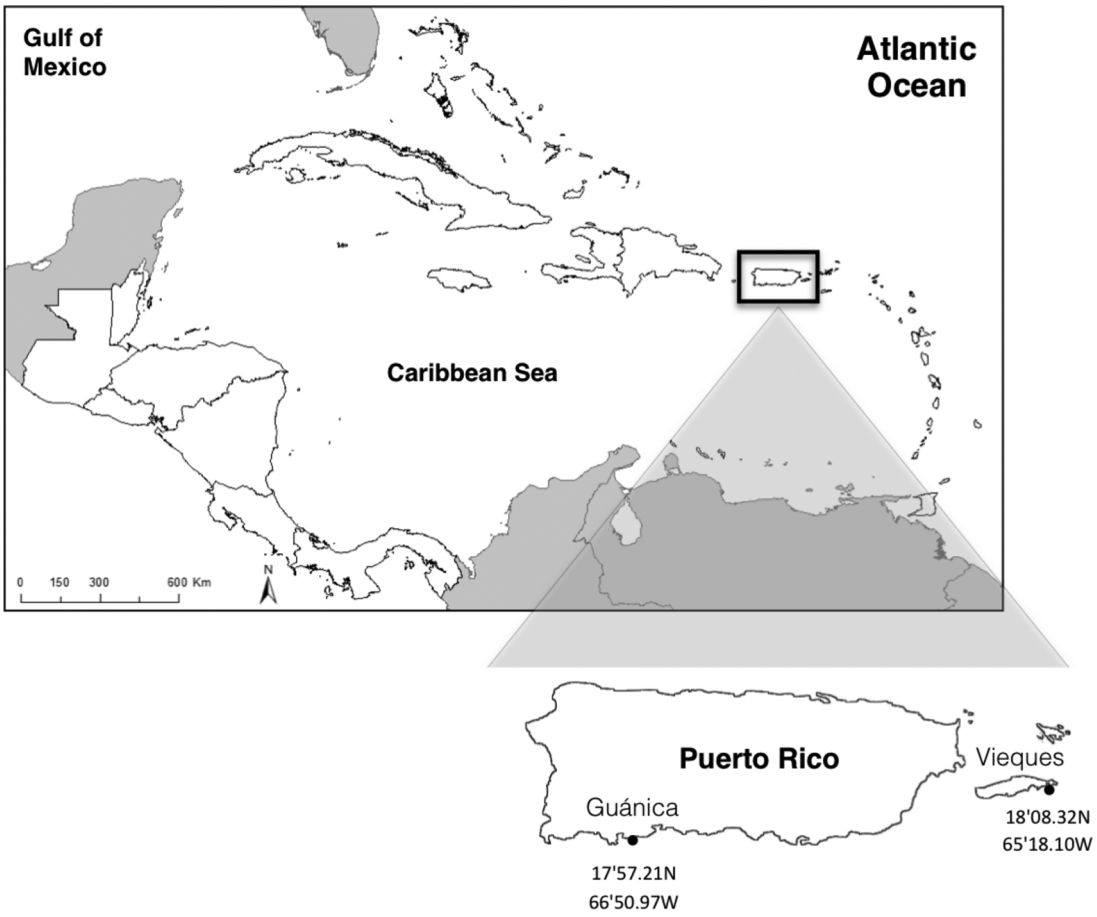

Fig. 1. Sampling site in Carrucho Beach at the former Atlantic Fleet Weapons Training Facility in Vieques, Puerto Rico. The reference location was located southwest mainland Puerto Rico in Tamarindo Beach at the Guánica State Dry Forest.

Zostera japonica to accumulate Pb, Cd and Cu to potentially enhanced environmental decontamination has been shown previously [11]. An increased concentration of Cd and Cu in seagrass increases oxidative stress and induces antioxidant defense systems against reactive oxygen species [12]. Furthermore, the accumulation of heavy metals by seagrasses can be a good indicator of a decrease of antioxidant levels due to free radicals [13]. Tolerance in plants to toxic elements has been extensively documented for over 35 years. Information on the ability of seagrass worldwide to absorb these elements is, however, still limited. The direct uptake of Pb, Cd, and Cu by aquatic plants increases the likelihood that the toxins will be transferred to marine organisms through the food web, including fish and endangered animals. For humans, it is known that prolonged consumption of fish contaminated with heavy metals can lead to biochemical disruption within organs, negatively impacting liver, kidney, cardiovascular, nervous and bone conditions [13,14].

In Vieques, a shallow bed of *Syringodium filiforme* is the dominant seagrass species along Carrucho Beach at the south coast of the former bombing range. Tribble [15] demonstrated that coral reef fish have a preference for *Syringodium* rather than other marine plants such as *Thalassia*. Their distribution in coral reefs is usually limited by selective grazing activity. Furthermore, this species is very common in the Caribbean and has been associated to high resistance to storm disturbances and perhaps to a variety of other environmental disturbances. Physiological differences and ecotypes of *S. filiforme* have been reported, including specific adaptation to light histories among others [16].

Because seagrasses represent a key species in the marine ecosystem, for 15 years we evaluated accumulation of Pb, Cd and Cu of acid digested samples from AFWTF and Guánica State Dry Forest (GSDF) as a reference location. These observations provide insights on the ecological consequences of anthropogenic disturbances and the potential transfer of pollutants through the open ecosystem.

2. Materials and methods

Our reference location to collect samples of *S. filiforme* was Tamarindo Beach at Guánica State Dry Forest (GSDF). It's geology and environmental conditions resemble those observed at the eastern part of Vieques;both sites are under the direct influence of the Caribbean Sea (Fig. 1). This forest encompasses great diversity, including endangered species, encompassing almost 1000 acres of land. Due to its ecological importance, it was designated in 1981 as a United Nations International Biosphere Reserve. Currently, this location is considered the best-preserved subtropical dry forest, as well as a great representative of dry forest in the Caribbean [17].

Samples of *S. filiforme* were manually collected from a one square meter plot at Carrucho Beach in the former AFWTF (18°08.32N, 65°18.10W) in the island of Vieques, Puerto Rico (Fig. 1). Replicate plots along the coastal line were sampled several times during 2001, 2003, 2005–2006, and 2013–2016. Samples were similarly taken from GSDF reference location at Tamarindo Beach (17°57.21N, 66°50.971W). After collection, samples were placed in large plastic bags and immediately transported to the laboratory. Samples were handled only with plastic, glass, or porcelain tools. Field and blank controls were included during sampling campaigns. Pb, Cd, and Cu concentrations were below detection limits in these controls.

Analyses of heavy metals followed Montgomery et al. [18] and Thompson [19]. Samples were rinsed thoroughly with deionized water, shaken to remove most of the water, allowed to air dry, and grounded in a ceramic mortar. Approximately 3 g of finely cut material was