Assessment of mangroves habitats by plant and soil enzymes studies in Boushehr province

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Abstract
Different studies were done for estimating of war pollution effect that happened in Persian Gulf in January 1991. In this study soil and plant enzymes determined the effect of pollution. In three different natural habitats (Assaluyeh, Bordekhoun and Dayyer) of Avicennia marina, fifteen healthy trees were selected. Branches related to each annual growth ring during 1989-2000 were separated for plant enzyme analysis by using mixture of same years from different trees in each habitats (as a regional indicator). The peroxidase analysis was done quantitatively and qualitatively by spectrophotometric and poly acrylamide gel electrophoresis (PAGE) methods, respectively. In addition soil sampling was done from different depths and transported in 4°C to laboratory. Four soil enzyme assay (Acid and alkaline phosphatase, dehydrogenase and β–D-glucosidase were done by use of special substrate and colorometric methods. There was conformity between soil and plant enzymes. Assaluyeh (a conserved habitat) showed no abnormality in soil enzyme and plant indicator gel pattern but plant indicator gel pattern of Bordekhoun and Dayyer habitats had abnormality. In addition results of soil enzymes indicated abnormal situation in two mentioned habitats. Results indicated in conserved ecosystems, resistance against pollution is more than exploited ecosystems. Results showed that tree could be used as suitable indicator for monitoring of pollution effects on natural ecosystems.

Key words: Mangrove, Soil l, Peroxidase, Phosphatase, Glucosidase, Dehydrogenase

Introduction
Following Persian Gulf war, huge amounts of atmospheric pollutants were distributed in the Persian Gulf region. Atmospheric and water pollutants penetrated in one third of Iran’s territory. In this study alteration of plants and soil enzymes was used for determining the effect of war pollution on Iranian natural ecosystems. Plants, especially trees have an important role in monitoring of environmental alteration (Ebermann et al., 1991; Korori et al., 2000; Shirvany et al, 2002;). Enzymes are the most important indicators in plants for environmental stress monitoring and peroxidase is one of the most sensitive enzyme for studying of ecological transitions (Castilo., 1990; Castilo, 1992; Ebermann and Stich, 1982; Ebermann and Stich, 1988; Ebermann et al., 1991; Korori, 1989; Pfanz and Opmann, 1991; Van Assche et. al., 1986). Based on the results of some research projects, amount of enzyme decrease from cambium towards the pith of trees, in the other hand more amount of cambium content leads to more activity per extracts (Ebermann and Stich., 1982; Ebermann and Stich., 1988; Stich, 1980).

Soil is a living system where, all biochemical activities proceed through enzymatic process. Enzymes are very important in modification of organic matter and show the quantity of life levels in soil systems (Castilo, 1990; Ebegt and Boldewijn, 1977). Based on many researches, soil enzyme activity gradient is the most important criteria for evaluation of
ecosystem (Gershuny and Smillie, 1995; Pimentel, 1995; Sachs, 1999; Van Assche et al., 1986). Among the many factors that may affect enzyme activities in soil, cropping history, soil amendments and some environmental factors have a special influence. Assay of dehydrogenase in surface soil indicated silviculutre and forestry practices such as clear cut had a negative effect on dehydrogenase activity and their effect was more in dry season (Quilcaano, and Mavanon, 2002). Activity enzymes were used for assessment of soil quality, (Bergstrom et al 1998). Results indicated soil enzyme activities changed according to slope and depth. Results of L-asparaginase-amidase and \( \beta \)-glucosidase activity assays showed that soil enzymes are very sensitive biological indicator for assessment of soil management (Miller and Richard, 1995). There was a relationship between hydrolase activity of soil and CO2 production, carbon biomass and ratio of carbon biomass to total carbon (Garcia et al., 1994).

In this study, the condition of plants bed were studied by use of soil enzymes and then effects of pollution on plants were monitored by use of enzymes alteration in plants.

Methods and Materials
- Study area
Bushehr province locates in south of Iran between 50\(^\circ\), 38\(^\prime\) - 51\(^\circ\), 21\(^\prime\) eastern longitude and 27\(^\circ\), 50\(^\prime\) - 29\(^\circ\), 40\(^\prime\) northern latituden from sea level to a maximum of 1950 meters. The plant cover is a mix of natural forests, rangelands and farmlands. The most important forest tree species are Avicennia marina, Ziziphus spina-christi, Prosopis cineraria, Acacia nilotica and shrubs such as Tecomella undolata, Leptadenia pyrotechnica, Ochradenus baccatus, Periploca aphylla, and Lycium spp. Sampling area located on natural mangrove forests locations in three regions including: Bordekhoun, Dayyer harbour and Assaluyeh (Nayband Gulf).

- Soil sampling
Soil sampling was done from different horizons of soil includes 0-10, 10-30, 30-40 cm. Samples were kept and transported back to the laboratory in 4\(^\circ\) C to the laboratory for enzymatic studies. Four soil enzymes were assayed including phosphatase (acid and alkaline), dehydrogenase and \( \beta \)-glucosidase (Dick, 2000; Evensong, 1982).

- Tree sampling:
In each region, 15 trees were selected and branches of related to each year during 1989-2000 were separated with an accurate procedure (Figure 1) and extracted for enzyme analysis. An equal amount of same year from fifteen stands mixed and extracted for enzymatic studies. This extract called regional indicator. Peroxidase alterations were studied qualitatively and quantitatively by PAGE and spectrophotometric methods (Korori, 1989).

Results
- Soil enzymes
Soil enzymes results include three sites in Bushehr province that apparently were affected by pollution directly and indirectly (as results of black rainfall). Figures 2-4 show soil enzyme alteration in three locations. Results indicated the normal pattern for all studied soil enzymes in Assaluyeh but in two other sites the patterns were abnormal. In Bordekhoun and Dayyer, only \( \beta \)-D-glucosidase and alkaline phosphatase had normal patterns, respectively. In the other hand, \( \beta \)-D-glucosidase and alkaline phosphatase (or microorganisms responsible for production of them) had less sensitivity against pollution in Bordekhoun and Dayyer. Assaluyeh ecosystem had a normal situation according to soil enzymes.

- Plant enzyme
Qualitative and quantitative alteration in regional indicator samples are shown in figures 5-10. Results indicated great qualitative alteration of peroxidase in Bordekhoun and Dayyer in
comparison to Assaluyeh during and after pollution period (almost 7 years). The range of quantitative alteration was more limited. These three locations had 60-Km distance of from each other. Isoenzymatic patterns indicated enzyme denaturation in seven years period (after the war) in Børdekhoun and Dayyer. In two mentioned locations, peroxidase showed qualitatively and quantitatively alterations as effect of war pollution. In Assaluyeh, gel pattern did not show abnormality. The negative effect of war pollution was only seen quantitatively in Assaluyeh.

Discussion
Persian Gulf war happened in January 1991. Researches have shown the extensive distribution of smoke and soot over the Persian Gulf region and surrounding countries. Reports of atmospheric pollutants reaching Turkey, Syria and as far as India were frequent. According to SCWMRC reports and based on analysis and processing of about 1267 NOVA AVHRR satellite pictures, about 35% of the total atmospheric pollutants resulting from burning of one billion barrels of Kuwait oil penetrated into Iran’s territory (Aminipouri et al., 1999).

In fact there were two forms of pollution. Ecosystems were affected by pollutant factors directly (as results of tide) and indirectly (black rainfall). Microorganism’s frequency (then their enzyme activity) in the upper layers of soils is more than the lower, then biological activity decrease in a vertical gradient (figure 11). Pollution effects on the soil biological texture lead microorganisms to move toward the lower horizons (Dick, 2000; Sachs, 1999). In order to estimate range of pollution effects on the several natural habitats, this hypothesis was used as a valuable tool for ecosystem evaluation. Results indicated that some of soil enzymes such as phosphatase (both alkaline and acid form) were affected by pollution severely. Soil enzyme results indicated the most abnormal alterations were observed in the Børdekhoun and Dayyer locations. These regions were selected as the most polluted regions based on the enzyme studies while industrial and transportation activities are more less in above-mentioned locations. Although Assaluyeh was located in pollution center but soil enzymatic pattern was normal in compared to Børdekhoun and Dayyer locations possibly due to ecosystem recovering potential against the pollution effects. As previously mentioned this location is a conserved area. According to ecosystem recovering potential and being conserved, normal peroxidase pattern was seen in regional indicator demonstrating resistance against pollution stress. Enzyme results in mangroves showed peroxidase denaturation in war year (1991) and several yeas after that in Børdekhoun and Dayyer regions whereas regional pattern in Assaluyeh trees had a normal condition Ebermann and Stich, 1982; Ebermann and Stich, 1988; Stich, 1980). Plant enzymatic results showed conformity with soil enzyme in the Børdekhoun and Dayyer regions. Also Assaluyeh location results indicated there wasn’t any abnormality both in the soil enzyme and regional plant indicator gel pattern (peroxidase qualitative studies). This may be due to ecosystem recovering potential against the pollution effects. Assaluyeh is a conserved ecosystem but two other regions are expolitated with different human activity such as fishing and branches harvesting. Denaturation of plant peroxidase may be as a result of war pollution. Abnormal pattern of soil enzyme may be partly due to war pollution or human activity. This study introduced usage of soil and plant enzyme alteration as a suitable method in assessment of ecosystem condition.

References
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Figure 1- sampling method scheme of trees for enzyme studied.
Figure 2- Soil enzymes alterations at the Bordekhoun location

Figure 2- Soil enzymes alterations at the Dayyer location
Figure 4- Soil enzymes alterations at the Assaluyeh location

Figure 5 - Bordekhoun gel image (regional indicator)
Figure 7 - Dayyer peroxidase gel image (regional indicator)
Figure 8 - Quantitative alteration of Dayyer complex extracts in peroxidase (Regional indicator)

Figure 9 - Assaluyeh peroxidase gel image (regional indicator)
Figure 10 - Quantitative activity of Assaluyeh complex extracts in peroxidase (Regional indicator)

Figure 11 - Scheme of soil enzyme study hypothesis.