Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 4, p. 65-73, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Profile of lead (Pb) heavy metal in water, sediment and seagrass (*Thalassia hemprichii*) in Ambon Island, Maluku, Indonesia

Charlotha I. Tupan[1], E. Y. Herawati[2], D. Arfiati[2], Aulanni'am[3]

[1]*Graduate School of Fishery and Marine Science, Brawijaya University, Malang, East Java, Indonesia and Lecturer at the Fishery and Marine Science Faculty, Pattimura University, Maluku. Jalan Veteran, Malang, 65145, Indonesia*

[2]*Faculty of Fishery and Marine Science, Brawijaya University, Malang, East Java. Jalan Veteran, Malang, 65145, Indonesia*

[3]*Faculty of Mathematics and Natural Science, Brawijaya University, Malang, East Java. Jalan Veteran, Malang, 65145, Indonesia*

Article published on October 12, 2014

Key words: *Thalassia hemprichii*, timbal, biomonitoring, bioindicator.

Abstract

The differences of lead heavy metal accumulation in water, sediment and among seagrass of *Thalassia hemprichii* (roots, rhizomes and leaves) were conducted on Ambon Island, Maluku. Samples were taken from two areas (Inner Ambon Bay and the southern part of the Ambon island) with a total of nine sampling sites based on seagrass distribution and land use. Pb was analyzed using Atomic Absorption Spectrophotometer. Pb metal content in the sediment is higher than the water and correlated significantly with the roots. The content of Pb on seagrass *T. hemprichii* showed a consistent pattern of accumulation at all the sites, where the Pb content in roots is higher than in the leaves and it is higher in the leaves than in the rhizomes. Thus the roots of the seagrass *T. hemprichii* can be used as bioindicators as well as biomonitoring of heavy metal pollution in the marine waters.

***Corresponding Author:** Charlotha I. Tupan ✉ lotjetupan@yahoo.com

Introduction

The pollution of coastal and marine environment has become a global issue, especially in the developed and developing countries. The pollution of coastal and marine generally occurs due to the concentration of population, industrialization and tourism activities in coastal areas. The impact of these activities on the coastal waters environment is likely to increase the amount of waste discharged into coastal waters. Heavy metals including hazardous waste serves as the sources of pollution, which is generally toxic in nature. Given their potential adverse effect to the environment and human health, these substances are thus of great concern to further studies (Rainbow, 2007; Roberts *et al*., 2008).

Metals can be found in the form of colloidal, particulate and dissolved form, although generally low concentrations of dissolved compounds (Kennish, 1992). Metals solubility in seawaters is controlled by salinity, pH, sediment type, ligand concentration, and redox potential (Guilizzoni 1991). The study of metal abundance in the water and sediments provide a relatively complete picture of the burden of contaminants in ecosystems, and various attempts have been made to evaluate metal pollution in water and sediments, including their interaction (Prego *et al*., 2006; Terrado *et al*., 2007; Abrahim and Parker, 2008). However, these approaches do not provide adequate information that is biologically relevant to ecotoxicology (Campanella *et al*., 2001). A suitable alternative for measuring the toxic chemical compounds, elements or their metabolites in tissue, called biomonitoring, giving the overall performance including integrated time availability of pollutants (Rainbow, 1995).

Biomonitoring has been widely applied to the study of metal contamination in algae and marine invertebrates, especially in mussels (Conti and Cecchetti, 2003; Rainbow, 2007). Seagrasses are marine angiosperms also suitable for metal biomonitoring (Pergent - Martini and Pergent, 2000; Ferrat *et al*, 2003; Ralph *et al*, 2006). Based on the fact that the seagrass grows abundant, widely distributed, has a long life time, and easily sampled and identified (Llagostera *et al*., 2011). In addition, seagrasses have high metal bioaccumulation capacity for interacting directly with the water column (through the leaves) and sediment (through the roots), therefore both leaves and roots are ion uptake sites (Romero *et al*., 2006; Ralph *et al*., 2006) so that it becomes a good bioindicator. The monitoring of heavy metal pollution in the water on the metal content in plant tissues showed high variability, the result of the interaction between bioavailability and plant physiology, including the kinetics of uptake and translocation between the internal parts of the plant, either passive or active. Therefore, the metal content can vary significantly among plant organs (leaf, rhizome and root). Thus, knowledge of the patterns of metal accumulation in different organs is very important to design the basic biomonitoring programs of seagrass and accurate interpretation of the data generated (Llagostera *et al*., 2011).

Seagrass as bioindicator studies have been done on the *Zostera marina* (Krause - Jensen *et al*., 2005), *Posidonia oceanica* (Romero *et al*., 2005; Gobert *et al*., 2009, Royo *et al*., 2010) *Cymodocea nodosa* (Oliva *et al*., 2011), and in Indonesian waters on the species of *Cymodocea rotundata* (Herawati, 2008), while the species of *T. hemprichii* still rarely investigated while its abundant existence and widespread distribution in the waters of Indonesia, especially on the Ambon island. With respect to the ability of seagrass to absorb and accumulate heavy metals Pb, it is necessary to investigate the heavy metal content of Pb in seawater, sediment and parts of roots, rhizomes and leaves of *T hemprichii* in the waters of Ambon Island which can be developed as a biomonitoring heavy metal pollution in sea water. The aim of this study was to identify differences in the accumulation of lead heavy metal in water, sediment and among the roots, rhizomes and leaves of the seagrass *T. hemprichii*.

Materials and method

Study Sites and Sampling

This research was conducted at the location of Ambon Bay (position 3^{o}38'30 "- 3^{o}39'30" S and 128^{o}11'30 "-

128º15'00" E) and the southern part of the Ambon island (position 3º42'00 "- 3º42'30" S and 128º16'00 "- 128º16'30" E). The selection of study sites of this present research is based on the Pb content, seagrass distribution, and land use. Based on previous studies by Mainassy *et al.,* (2005), heavy metals Pb in the Ambon Bay in the range between 0.03 to 0.38 mg/l which Poka and Lateri location are an area of high Pb contaminated. Poka and Lateri also have the distribution of seagrass *T. hemprichii* that is one of the dominant species (Tuhumury, 2008). Therefore Poka and Lateri become the location of the study site in the Ambon Bay. Poka consists of: Site 1 (P1); the area around the row-row ship wharves, site 2 (P2); residential areas, and site 3 (P3) the territory diesel. Lateri, consist of: site 1 (L1); the area around sedimentation, site 2 (L2); residential areas, and station 3 (L3) the mangrove area. The location of the southern part of the Ambon island is visually clean and there are no data of heavy metal content previously. The location which was distributed by abundance of seagrass in this region is the Rutong area and *T. hemprichii* is one of the dominant species (Tupan, 2012). Rutong area, consists of: sites 1 (R1) the area near the river, site 2 (R2) residential area and site 3 (R3) mangrove area.

Seagrass sampling was conducted in November 2011 at each site, where the distance between sites 300-500 meters. At each site, seagrass was colected three times from an area of 1m^2 separated by 25-50 m. Each samples consisted of 30 shoots, including leaves, rhizomes and roots. Shoots were rinsed with seawater *in situ* to remove sediment particles and then packed in plastic bags, and transported to the laboratory. Water and sediment sampling was also conducted on seagrass sampling area.

Analysis of Lead (Pb) Heavy Metal

For the preparation of metal analysis, seagrass organs were washed again with sea water to remove residual sediment and other debris. Rinsing seagrass with distilled water resulted in a premature leaching of metals and other cations (Ledent *et al*., 1995). Epiphytes were removed from the leaves by scraping with hand. Seagrass organs were dried to constant weight at a room temperature, then dried in the oven at 100ºC for 5 h. Samples were grounded in an mortar to obtain a homogenous powder, then weighed as much as 1 g and added as many as 5 ml of 5 M HNO_3, stirred and heated with a hot plate at 85-95ºC. The samples were allowed to cool and filter into 50 ml volumetric flask using Whatman no 5 filtered paper, then added distilled water to mark the limits. After that, samples are ready to analyze by means of Atomic Adsorption Spectrophotometer (AAS) at a wave length of 283.3 nm. Method and instrument used for extraction and lead analysis of sediment and sea water are similar to those used for seagrass organs.

Data analysis

The data were analysed using ANOVA statistical analysis on the 5% significance level to test for differences in the content of heavy metal Pb (dependent variable) among nine study sites and among three factors seagrass (roots, rhizomes and leaves) (independent variables) and their interaction. If there is a difference, then followed by least significant difference test (LSD) at the 5% significance level. Prior to analysis, first had gone through the data normality test using Kolgomorov - Smirnov test and homogeneity test using Levene's test and Bartlett's test, and the test of non additivity as a requirement for ANOVA test. The test results concluded that the observational data qualify normality, homogeneity and non-additivity, so it can proceed to ANOVA test. Finally, we explored the quantitative consistence in the pattern of accumulation of Pb metal between the different organs (roots, rhizomes, leaves) and environment (water and sediment).

Results

Pb metal content in water and sediment

The metal content of Pb in water and sediment from the nine study sites shown in Fig. 1. There were significant differences in the Pb content of water and sediment based on ANOVA (Table 1). There was significant spatial variability, significant between water and sediment and interaction. There are contributions from each source of variability. Spatial variations (among sites) provide 11% of variability, where P1 has the smallest Pb content and are very different from R1 and R2, and R2 has the highest Pb contents (Fig. 1). Furthermore, variations between water and sediment provide variability by 59% where sediments have much higher Pb contents than the water. Based on the correlation analysis, there was no correlation between water and sediment (Table 3).

Pb metal content on roots, rhizomes and leaves

The metal content of Pb on seagrass *T. hemprichii* (roots, rhizomes and leaves) can be seen in Fig. 2. Based on ANOVA, there were significant differences between sites and organs, while the interaction between the site and the organs are not significantly different (Table 2). Spatial variation (among sites) explained 13% of variability which Pb content of the L3 site has the smallest, while site R1 has the highest Pb content (Fig. 2). Pb content in organs provide variability by 37%, where the roots have the highest content of Pb followed by leaves, while the rhizome has a low Pb contents (Fig. 2). Based on the correlation analysis it is known that the pair sediment and roots, sediment and rhizomes, roots and rhizomes and rhizome and leaves correlated significantly (Table 3).

Table 1. Result of ANOVA (site: 9 levels and environment: 2 levels) and LSD analysis for factor environment. Significance level settled at 0.05.

Metal	Source of variation	p-Value	% Variability	Post hoc
Pb	Site	0.022	11	
	Environment	0.000	59	Sediment > water
	Site x environment	0.022	11	
	Error		19	

Table 2. Result of ANOVA (site: 9 levels and organ: 3 levels) and LSD analysis for factor organ. Significance level settled at 0.05.

Metal	Source of variation	p-Value	% Variability	Post hoc
Pb	Site	0.042	13	
	Organ	0.000	37	Roots > leaves > rhizomes
	Site x organ	0.512	11	
	Error		39	

Table 3. Result of correlation analysis between Pb metal content in water, sediment and *T. hemprichii* (roots, rhizomes and leaves). (r is the correlation coefficient). Significance level settled at 0.05. Bold numbers indicates significant correlation.

Metal	Water-sediment		Water-roots		Water-rhizomes		Water-leaves	
	r	p-Value	r	p-Value	r	p-Value	r	p-Value
Pb	-0.0231	0.9088	-0.0301	0.8813	-0.2364	0.2351	0.0349	0.8627

Metal	Sediment-roots		Sediment-rhizomes		Sediment-leaves	
	r	p-Value	r	p-Value	r	p-Value
Pb	0.5682	**0.0020**	0.4566	**0.0167**	0.1029	0.6096

Metal	Roots-rhizomes		Roots-leaves		Rhizomes-leaves	
	r	p-Value	r	p-Value	r	p-Value
Pb	0.4658	**0.0143**	0.3744	0.0543	0.5194	**0.0055**

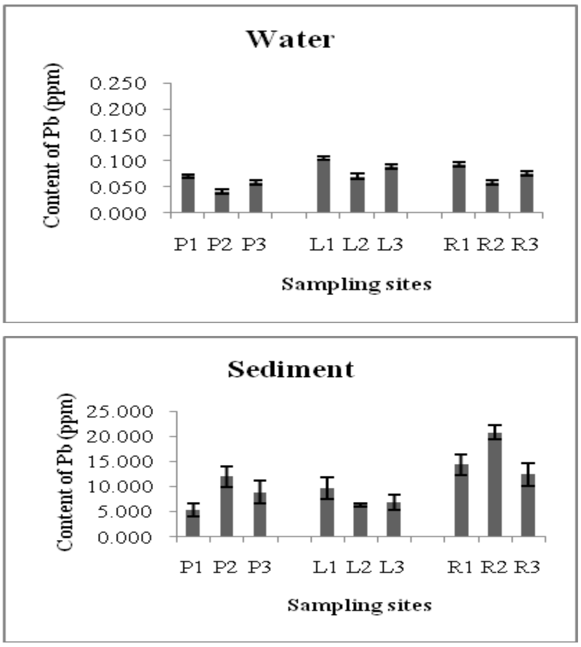

Fig. 1. Pb content in water and sediment.

Fig. 2. Pb content in roots, rhizomes and leaves of *T. hemprichii*.

Discussion

Pb metal content in water and sediment

Pb heavy metal is one type of metal that is commonly found in coastal waters due to the utilization of fuel and industrial paints are often used by ships. Pb was found in all the study sites in the waters of Ambon Island, although its concentration is lower than the one found by Mainassy *et al.*, (2005) in the Ambon Bay. Pb content of these waters are also much lower than Pb content in the sediment. Activity coastal communities that contribute to the water content of Pb in the sediment accumulates more stout from those of the water column, so that the sediment is more representative to record the accumulation of heavy metals in waters. The solubility of metal elements and heavy metals in waters controlled by the pH of the water column (Guilizzoni, 1991). Low pH or acidic can dissolve heavy metals, and alkaline pH would affect heavy metal for not dissolve. pH on Poka waters ranged from 7.51 to 7.82, Lateri waters ranged from 7.27 to 7.69 and Rutong waters ranged from 7.80 to 8.10. The range of this pH is alkaline, so it affects metals Pb difficult soluble and settles in the bottom of the waters. Heavy metals dissolved in the water will also move into the sediments if bound to free organic matter or organic matter that lines the surface of the sediment, and direct absorption by the surface of the sediment particles, due to heavy metal binding organic materials easily so that the heavy metal content in the sediment was higher than in the water (Wilson, 1988).

Pb content of the sediment has the highest on Rutong waters because there is bottom substrate dominated by coral rocks thought to be one source of Pb on Rutong sea waters. Sources Pb naturally derived from the release of metals from mineral rocks due to waves and wind (Palar, 2008). Rutong waters with high waves in certain time allow the release of metals in coral rock. In addition, the waters affected by the banda sea volcano allow the upwelling that also contribute of Pb to these waters.

Pb metal content on the roots, rhizomes and laves of T. hemprichii

The presence of Pb on seagrass *T. hemprichii* obtained directly from the water through absorption by the roots of the sediment and the leaves from the water column. According to Romero *et al.,* (2006) and Ralph *et al.*, (2006) that seagrass has a high capacity to accumulate heavy metals because it interacts directly with the water column through the leaves and the sediments through the roots. In this study the Pb accumulation pattern is fairly consistent at all sites where the content of Pb in the roots is higher than in the leaves, and the leaves is higher than in the rhizome. The content of Pb in the roots was higher than the leaves and rhizome because the roots absorb Pb directly from the sediments that also accumulated Pb contents were very high. Pb content which are higher in roots than leaves is also found in the seagrass *Zostera capricorni* (Ambo Rappe, 2008) and *Cymodocea nodosa* (Liagostera *et al.*, 2011). In addition, Marin-Guirao *et al.*, (2005) have found higher content of Pb in the roots in comparison with the leaves of *Cymodocea nodosa*. They explain that the leaves of *C. nodosa* have absorbed Pb from the water when the availability of Pb in sediments is low. Guilizzoni (1991) has pointed out that aquatic angiosperms have extracted nutrients and heavy metals mostly from sediments via root hairs, with subsequent translocation to the upper parts. He has described that most aquatic species have a well-developed vascular system; as a result, basipetal and acropetal water flow may transport ions through the xylem.

Metal accumulation in seagrass organ has been reported by several authors, such as Pergent-Martini (1998) and Pergent and Pergent-Martini (1999) have observed high Hg accumulation in the leaves of Posidonia oceanica, and interpreted as an indication of high level of heavy metal contamination in the water column at the sampling sites. Alvares - Legoreta *et al.*, (2008) has shown that the Cd metal in seawater is concentrated in the leaves of *Thalassia testudinum* and transported through basipetal translocation to the root system. Seagrass organs differences in the absorbing metal, according Llagostera *et al.,* (2011) is influenced by three factors: 1) the availability of metals in the water column and sediments differ from one location to another depending on the metal supply, 2) Both the uptake kinetics and passive absorption properties of leaves may differ from those of the root, and 3) subsequent metal uptake can be distributed internally through active or passive transport mechanisms.

Significant correlation between sediment and seagrass *T. hemprichii* at the root and rhizome showed that sediment is very representative to describe the Pb accumulation in plant organs, especially seagrass roots, because roots absorb Pb directly from the sediment. Besides that, the pattern of accumulation of Pb on seagrass *T. hemprichii* is supported by a significant correlation between the sediment and roots, root and rhizome and the rhizome and leaves showed that the organs in particular seagrass roots can be selected to fit the criteria of choice biomonitoring organ that has a high concentration (Llagostera *et al.*, 2011), where the roots are very representative in the record of Pb contaminants in the waters through the sediment.

Conclusion

The results of Pb heavy metal content in the water, sediment and seagrass organs (roots, rhizomes, leaves) of *T. hemprichii* in the waters of Ambon Island have shown that the accumulation of Pb is mostly found in sediment and seagrass root. Sediment is considered to be very representative to describe the total load of the water pollution. Additionally, the concentration of Pb heavy metal and its accumulation pattern in this seagrass organ may reflect the concentration and the bioavailability of the Pb heavy metal in Ambon Island waters. Thus, *T. hemprichii* may have application as indicator organism as well as biomonitoring of Pb heavy metal contamination and bioavailability in seawater.

Acknowledgments

We are grateful to Director Generals of Higher Education, Ministry of Education and Culture of

Republic of Indonesia for funding this study and research. Our best gratitude to the Chief and Staffs of Environment Laboratory, Chemistry Department, School of Mathematics and Physics Brawijaya University for analyzing Pb heavy metal.

References

Abrahim GMS, Parker RJ. 2008. Assessment of heavy metal enrichment factors and the degree of contamination in marine sediments from Tamaki Estuary, Auckland, New Zeland. Environmental Monitoring and Assessment **136**, 227–238.

Alvares-Legorreta T, Mendoza-Cozalt D, Moreno Sanchez R, Gold Boichot G. 2008. Thiol Peptides induction in the seagrass Thalassia testudinum (Banks ex Konig) in response to cadmium exposure. Aquatic Toxicology **86**, 12-19

Ambo Rappe R. 2008. Heavy Metals Accumulation and Possible Translocation in Seagrass, Zostera capricorni Aschers. Torani **18 (2)**, Jurnal Ilmu Kelautan dan Perikanan Universitas Hasanuddin. Makasar.

Campanella L, Conti ME, Cubadda F, Sucapane C. 2001. Trace metals in seagrass, algae and molluscs from an uncontaminated area in the Mediterranean. Environmental Pollution **111**, 117–126.

Conti ME, Cecchetti G. 2003. A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. Environmental Research **93**, 99–112.

Ferrat L, Pergent-Martini C, Romeo M. 2003. Assessment of the use of biomarkers in aquatic plants for the evaluation of environmental quality: application to seagrasses. Aquatic Toxicology **65**, 187–204.

Gobert S, Sartoretto S, Rico-Raimondino V, Andral B, Chery A, Lejeune P, and Boissery P. 2009. Assessment of The Ecologycal Status of Mediterranean French Coastal Waters as Required by The Water Framework Directive Using The Posidonia oceanica Rapid Easy Index: PREI. Marine Polution Bulletin **58 (11)**, 1727-1733

Guilizzoni P. 1991. The role of heavy metals and toxic materials in the physiological ecology of submersed macrophytes. Aquatic Botany **41**, 87–109.

Herawati EY. 2008. Lamun (Cymodocea rotundata, Thalassia hemprichii dan Enhalus acoroides) Sebagai Bioindikator Logam Berat Timbal (Pb) di Perairan Pesisir Jawa Timur. Disertasi. Ilmu Pertanian. Program Pascasarjana. UB. Malang.

Kennish MJ. 1992. Ecology of Estuaries: Anthropogenic Effects. CRC Press, Boca Raton, FL.

Krause-Jensen D, Greve TM and Nelsen K. 2005. Eelgrass as a Bioindicator Under The European Water Framework Directive. Water Resources Management. **19**, 63-75

Ledent G, Mateo MA, Warnau M, Temara A, Romero J, Dubois Ph. 1995. Element losses following distilled water rinsing of leaves of the seagrass Posidonia oceanica (L) Delile. Aquatic Botany **52**, 229-235

Lewis MA, Devereux R. 2009. Non nutrient anthropogenic chemicals in seagrass ecosystem: fate and effects. Environmental Toxicology and Chemistry **28**, 644-661

Llagostera I, Perez M, and Romero R. 2011. Trace Metal Content in The Seagrass Cymodocea nodosa: Differential accumulation in plant organs. Aquatic Botany **95**, 124 – 128.

Mainassy B, Huliselan NV, Tuhumury SF dan Wattimury JJ. 2005. Penentuan Lokasi Pengembangan Budidaya Keramba Jaring Apung (KJA) di Perairan Teluk Ambon Dalam (TAD) Menggunakan Sistem Informasi Geografis. Ichthyos **4 (2)**, 69-80. Fakultas Perikanan dan Ilmu Kelautan. UNPATTI. Ambon

Marin-Guirao L, Marin A, Lioret J, Martinez E, Garcia AJ. 2005. Effect of minning wastes on a seagrass ecosystem: metal acumulation and bioavilabillity, seagrass dinamics and associated community structure. Marine Environmental Research **60**, 317-367.

Oliva S, Mascaro O, Llagostera I, Perez M, and Romero J. 2011. Selection of Metrics on The Seagrass *Cymodocea nodosa* and Development of a Biotic Index (CYMOX) for Assessing Ecological Status of Coastal and Transitional Waters. Estuarine, Coastal and Shelf Science **xxx**, 1-11

Palar H. 2008. Pencemaran dan Toksikologi Logam Berat. PT Rineka Cipta. Jakarta

Pergent G, Pergent-Martini C. 1999. Mercury levels in Posidonia oceanica meadows. Environmental Pollution **106**, 33-37

Pergent-Martini C. 1998. Posidonia oceanica: a biological indicator of plant and present mercury contamination in the Mediteranean Sea. Marine Environmenal Research **45**, 101-111

Pergent-Martini C, Pergent G. 2000. Marine phanerogams as a tool in the evaluation of marine trace-metal contamination: an example from the Mediterranean. Environmental Pollution **13**, 1– 6.

Prego R, Cotte MH, Cobelo-Garcia A, Martin JM. 2006. Trace metalsin the water column of the Vigo Ria: offshore exchange in mid-winter condition. Estuarine Coastal and Shelf Science **68**, 289-296

Rainbow PS. 1995. Biomonitoring of heavy metal availability in the marine environment. Marine Pollution Bulletin **31**, 183–192.

Rainbow PS. 2007. Trace bioaccumulation: models, metabolic availability and toxicity. Environment International **33,** 576-582.

Ralph PJ, Tomasco D, Moore K, Seddon S, Machinnis-Ng CMO. 2006. Human impact on seagrasses: eutrophication sedimentation and contamination. In: Larkum, A.W.D., Orth, R.J., Duarte, C (eds), Seagrasses: Biology, Ecology and Conservation. Springer, pp.567-593.

Roberts DA, Johnston EL, Poore AGB. 2008. Contamination of marine biogenic habitats and effect upon associated epifauna. Marine Pollution Bulletin **56,** 1057-1065.

Romero J, Alcoverro T, Crego BM and Peres M. 2005. The Seagrass Posidonia oceanica as a Quality Element Under The water Framework Directive: POMI, a Multivariate Method to Assess Ecological Status of Catalan Coastal Waters. Working document of the POMI group, University of Barcelona and Centre d'Estudis Avancats de Blanes (CSIC). 15 pp.

Romero J, Lee KS, Perez M, Mateo MA, Alcoverro T. 2006. Nutrient dynamics in seagrass ecoystems. In: Lrakum, A.W.D., Orth, R.J., Duarte, C. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer, pp. 227–254.

Royo CLY, Casazza G, Pergent-Martini C, Pergent G. 2010. A Biotic Index Using The Seagrass *Posidonia oceanica* (BiPo), to Evaluate Ecological Status of Coastal Waters. Ecologycal Indicators **10**, 380-389.

Terrado M, Kuster M, Raldua D, Lopez de Alba M, Barcelo D, Tauler R. 2007. Evaluation of pesticides pollution in the irrigation and drainage channels of the Ebro river delta during the growing season of rice using chemometric and geostatistical methods. Analytical and Bioanalytical Chemistry **387**, 1479-1488

Tuhumury SF. 2008. Status Komunitas Lamun di Perairan Pantai Teluk Ambon Dalam. *Ichthyos*. Jurnal Penelitian Ilmu-Ilmu Perikanan dan Kelautan **7 (2)**, 85-88

Tupan Ch I. 2012. Status Komunitas Lamun di Perairan Pantai Rutong, Bagian Selatan Pulau Ambon, Maluku. Proceeding. Seminar Nasional Teori dan Aplikasi Teknologi Kelautan. Fakultas Teknologi Kelautan. ITS. Surabaya

Willson JG. 1988. The biology of estuarine management . St Edmundsbury Press Ltd. Suffolk. Great Britain.