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Table of contents

Volume 55

2017

◆ Previous issue Next issue ➤

2nd International Conference on Tropical and Coastal Region Eco Development 2016 25–27 October 2016, Bali, Indonesia

Accepted papers received: 30 January 2017

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Preface OPEN ACCESS 011001 2nd International Conference on Tropical and Coastal Region Eco Development 2016 + Open abstract View article 🔼 PDF **OPEN ACCESS** 011002 Peer review statement View article 🔼 PDF + Open abstract **Papers OPEN ACCESS** 012001 Past and Future Ecosystem Change in the Coastal Zone P Gell + Open abstract View article 🔼 PDF

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012002

Conserving endangered marine organisms: causes, trends and challenges

Ambariyanto

+ Open abstract

View article

🔁 PDF

OPEN ACCESS 012003

Population of *Aedes sp* in Highland of Wonosobo District and Its Competence as A Dengue Vector

Martini Martini, Bagoes Widjanarko, Retno Hestiningsih, Susiana Purwantisari and Sri Yuliawati

+ Open abstract

View article

PDF

OPEN ACCESS 012004

Nutritional value content, biomass production and growth performance of *Daphnia magna* cultured with different animal wastes resulted from probiotic bacteria fermentation

Vivi Endar Herawati, R A Nugroho, Pinandoyo and Johannes Hutabarat

+ Open abstract

View article



OPEN ACCESS 012005

Symbiotic Fungus of Marine Sponge Axinella sp. Producing Antibacterial Agent

A Trianto, S Widyaningsih, OK Radjasa and R Pribadi

+ Open abstract

View article



OPEN ACCESS 012006

The Influence of Madden Julian Oscillation on the Formation of the Hot Event in the Western Equatorial Pacific

Anindya Wirasatriya, Denny Nugroho Sugianto and Muhammad Helmi

+ Open abstract





OPEN ACCESS 012007

Sediment Transport Model In Sayung District, Demak

Aris Ismanto, Muhammad Zainuri, Sahala Hutabarat, Denny Nugroho Sugianto, Sugeng Widada and Anindya Wirasatriya

+ Open abstract





OPEN ACCESS 012008

Resources Management Strategy For Mud Crabs (Scylla spp.) In Pemalang Regency

Aristi Dian Purnama Fitri, Herry Boesono, Agus Sabdono and Nadia Adlina

+ Open abstract





OPEN ACCESS 012009

Development of Spore Protein of *Myxobolus koi* as an Immunostimulant for Prevent of Myxobolusis on Gold Fish (*Cyprinus carpio* Linn) by Oral Immunisation

Gunanti Mahasri

+ Open abstract

View article

PDF

OPEN ACCESS 012010

The Effectiveness of Extracts Basil Leaves (*Ocimum sanctum* Linn) against *Saprolegnia* sp. by in Vitro

Sudarno, Muhammad Luthfi Hakim and Rahayu Kusdarwati

+ Open abstract

View article

PDF

OPEN ACCESS 012011

Developing groundwater conservation zone of unconfined aquifer in Semarang, Indonesia

T T Putranto, W K Hidajat and N Susanto

+ Open abstract

View article

PDF

OPEN ACCESS 012012

Understanding coastal processes to assist with coastal erosion management in Darwin Harbour, Northern Territory, Australia

S.G. Tonyes, R.J. Wasson, N.C. Munksgaard, K.G. Evans, R. Brinkman and D.K. Williams

+ Open abstract

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OPEN ACCESS 012013

Environmental and Risk Factors of Leptospirosis: A Spatial Analysis in Semarang City

Silviana Nur Fajriyah, Ari Udiyono and Lintang Dian Saraswati

+ Open abstract



🔁 PDF

OPEN ACCESS 012014

Study of inundation events along the southern coast of Java and Bali, Indonesia (case studies 4-9 June 2016)

I R Nugraheni, D P Wijayanti, D N Sugianto and A Ramdhani

+ Open abstract



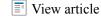
🔁 PDF

OPEN ACCESS 012015

The Use of Water Exchange for Feeding Rate and Growth Promotion of Shortfin Eel *Anguilla bicolor bicolor* In Recirculating Water System

N Taufiq-Spj, S Sunaryo, A Wirasatriya and D N Sugianto

+ Open abstract



🔁 PDF

OPEN ACCESS 012016

ASSOCIATION BETWEEN MACROMINERALS INTAKE AND CHANGES IN INTERNAL CAROTID ARTERY-INTIMA MEDIA THICKNESS IN POST ISCHEMIC STROKE PATIENTS

Dwi Pudjonarko, Dodik Tugasworo and Rumintang Silaen

+ Open abstract ☐ View article ☐ PDF

OPEN ACCESS 012017

Brain Gym To Increase Academic Performance Of Children Aged 10-12 Years Old (Experimental Study in Tembalang Elementary School and Pedalangan Elementary School Semarang)

M G Marpaung, T P Sareharto, A Purwanti and D Hermawati

OPEN ACCESS 012018

Polymeric Membrane Made of Cellulose Isolated from Tropical Water Hyacinth Blended with Chitosan

Titik Istirokhatun, Richa Rachmawaty, Metty Meriyani, Nur Rokhati and Heru Susanto

OPEN ACCESS 012019

Identification and Antibacterial Activity of Bacteria Isolated from Marine Sponge *Haliclona* (*Reniera*) sp. against Multi-Drug Resistant Human Pathogen

Meezan Ardhanu Asagabaldan, D Ayuningrum, R Kristiana, A Sabdono, O K Radjasa and A Trianto

+ Open abstract■ View articlePDF

OPEN ACCESS 012020

COPE Method Implementation Program to Reduce Communication Apprehension Level in Full Day Yunior High School Students

A R Prasetyo

+ Open abstract■ View article▶ PDF

OPEN ACCESS 012021

Influence of ENSO and IOD to Variability of Sea Surface Height in the North and South of Java Island

Ahmad Fadlan, Denny Nugroho Sugianto, Kunarso and Muhammad Zainuri

+ Open abstract■ View article▶ PDF

OPEN ACCESS 012022

The Roles of Macrobenthic Mollusks as Bioindicator in Response to Environmental Disturbance : Cumulative *k*-dominance curves and bubble plots ordination approaches

Sapto P. Putro, Fuad Muhammad, Amalia Aininnur, Widowati and Suhartana

+ Open abstract View article 🔁 PDF **OPEN ACCESS** 012023 The Effect of Early Mosquito Insecticides Exposure on Sprague Dawley Rat Testis: A Histopathological Feature Towards Malignancy? Tri Indah Winarni, Milzam Auzan Aziman, Anindyo Abshar Andar and Ika Pawitra View article 🔼 PDF + Open abstract **OPEN ACCESS** 012024 Analysis of heavy metal content of Cd and Zn in ballast water tank of commercial vessels in Port of Tanjung Emas Semarang, Central Java Province A Tjahjono, A N Bambang and S Anggoro + Open abstract View article 🔼 PDF **OPEN ACCESS** 012025 Proteomics study of extracellular fibrinolytic proteases from *Bacillus licheniformis* RO3 and Bacillus pumilus 2.g isolated from Indonesian fermented food Diana Nur Afifah, Ninik Rustanti, Gemala Anjani, Dahrul Syah, Yanti and Maggy T. Suhartono View article 🔼 PDF + Open abstract **OPEN ACCESS** 012026 Screening Antibacterial Agent from Crude Extract of Marine-Derived Fungi Associated with Soft Corals against MDR-Staphylococcus haemolyticus A Sabdaningsih, O Cristianawati, M T Sibero, H Nuryadi, O K Radjasa, A Sabdono and A Trianto View article 🔼 PDF + Open abstract **OPEN ACCESS** 012027 Exploration of Fungal Association From Hard Coral Against Pathogen MDR Staphylococcus haemolyticus O Cristianawati, O K Radjasa, A Sabdono, A Trianto, A Sabdaningsih, M T Sibero and H Nuryadi View article 🔁 PDF + Open abstract **OPEN ACCESS** 012028 Isolation, Identification And Screening Antibacterial Activity from Marine Sponge-Associated Fungi Against Multidrug-Resistant (MDR) Escherichia coli Mada Triandala Sibero, Aninditia Sabdaningsih, Olvi Cristianawati, Handung Nuryadi, Ocky Karna Radjasa,

+ Open abstract

Agus Sabdono and Agus Trianto

View article

🔼 PDF

OPEN ACCESS 012029 Isolation, Characterisation and Antagonistic Activity of Bacteria Symbionts Hardcoral Pavona sp. Isolated from Panjang Island, Jepara Against Infectious Multi-drug Resistant (MDR) Bacteria D. Ayuningrum, R. Kristiana, M.A. Asagabaldan, A. Sabdono, O.K. Radjasa, H. Nuryadi and A. Trianto View article 🔁 PDF + Open abstract OPEN ACCESS 012030 Encapsulation of phycocyanin-alginate for high stability and antioxidant activity Hadiyanto, Meiny Suzery, Deny Setyawan, Dian Majid and Heri Sutanto + Open abstract View article 🔼 PDF **OPEN ACCESS** 012031 The Correlation of Upwelling Phenomena and Ocean Sunfish Occurrences in Nusa Penida, C K Tito and E Susilo 🔼 PDF + Open abstract View article **OPEN ACCESS** 012032 Initial Study Of Potency Thermal Energy Using OTEC (Ocean Thermal Energy Conversion) As A Renewable Energy For Halmahera Indonesia Y O Andrawina, D N Sugianto and I Alifdini + Open abstract View article 🔼 PDF **OPEN ACCESS** 012033 Effect of Reaction Temperature on Biodiesel Production from Chlorella vulgaris using CuO/Zeolite as Heterogeneous Catalyst Dianursanti, M Delaamira, S Bismo and Y Muharam View article 🔼 PDF + Open abstract **OPEN ACCESS** 012034 Annona muricata modulate brain-CXCL10 expression during cerebral malaria phase Kis Djamiatun, Sumia M A Matug, Awal Prasetyo, Noor Wijayahadi and Djoko Nugroho View article 🔁 PDF + Open abstract **OPEN ACCESS** 012035 SOCIO-ECONOMIC SPATIAL FOR THE SUSTAINABILITY OF THE ESTUARY ECOSYSTEM IN PELABUHANRATU COASTAL WEST JAVA L. Supriatna, J. Supriatna and D. Harmantyo View article 🔁 PDF + Open abstract

OPEN ACCESS	012036
The Effect of One-to-one Counseling to Pregnant Women's Knowledge about Anemia in Semarang	
N P R Egryani, F Saktini, N Susilaningsih, V D Puspitasari and A R Gumay	
+ Open abstract ■ View article PDF	
OPEN ACCESS	012037
Nutrient Content And Acceptability Of Snakehead-Fish (<i>Ophiocephalus Striatus</i>) And Pumpkin (<i>Cucurbita Moschata</i>) Based Complementary Foods	
Etika Ratna Noer, Aryu Candra and Binar Panunggal	
+ Open abstract	
OPEN ACCESS	012038
Isolation and Identification of <i>Aeromonas hydrophila</i> and <i>Saprolegnia</i> sp. on Catfish (<i>Clarias gariepinus</i>) in Floating cages in Bozem Moro Krembangan Surabaya	
Rahayu Kusdarwati, Kismiyati, Sudarno, Hendi Kurniawan and Yudha Teguh Prayogi	
+ Open abstract	
OPEN ACCESS The Thermocline Layer and Chlorophyll-a Concentration Variability during Southeast Monsoon in the Banda Sea	012039
Nikita Pusparini, Budi Prasetyo, Ambariyanto and Ita Widowati	
♣ Open abstract ▼ View article PDF	
OPEN ACCESS Identification of wave energy potential with floating oscillating water column technology in Pulau Baai Beach, Bengkulu	012040
I Alifdini, D N Sugianto, Y O Andrawina and A B Widodo	
+ Open abstract	
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Retno Hartati, Agus Trianto and Widianingsih	
♣ Open abstract Image: View article PDF	
OPEN ACCESS Social Relation between Businessman and Community in Management of Intensive Shrimp Pond Indra Gumay Febryano, James Sinurat and Messalina Lovinia Salampessy	012042

7/26/2020

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Designing Cognitive Intervention to Improve the Awareness Index of the Residents in the Landslide Area

- N. Susanto, T. T. Putranto and E. A. Ulfa
- + Open abstract
- View article
- 🔼 PDF

OPEN ACCESS 012044

Genetic diversity of the causative agent of ice-ice disease of the seaweed Kappaphycus alvarezii from Karimunjawa island, Indonesia

E Syafitri, S B Prayitno, W F Ma'ruf and O K Radjasa

- + Open abstract
- View article
- 🔼 PDF

OPEN ACCESS 012045

Capacity Building Resource Management Of Coastal Areas To Improve The Local Economic Based By Cross-Cutting Partnerships: Case Study on Panjang Beach Bengkulu City

Titi Darmi

- + Open abstract
- View article
- 🔼 PDF

OPEN ACCESS 012046

The Dynamics of Sea Surface Height and Geostrophic Current in the Arafura Sea

Umaroh, Sutrisno Anggoro and Muslim

- + Open abstract
- View article
- 🔼 PDF

OPEN ACCESS 012047

Vulnerability Assessment: The Role of Coastal Informal Settlement Growth to Social Vulnerability in Genuk Sub-District, Semarang City

Sariffuddin, Khristiana Dwi Astuti, Gustika Farhaeni and Lutfiyatul Wahdah

- + Open abstract
- View article
- PDF

OPEN ACCESS 012048

Waves Induce Sediment Transport at Coastal Region of Timbulsloko Demak

Purnomo Hawati, Denny Nugroho Sugianto, Sutrisno Anggoro, Anindya Wirasatriya and Sugeng Widada

- + Open abstract
- View article
- 🔼 PDF

OPEN ACCESS 012049

Potential impact of climate variability on respiratory diseases in infant and children in Semarang

•	ati, S P Jati and P Gina	_	
+ Open abstract	☐ View article	PDF	
OPEN ACCESS			012050
Early Childhood	Environmental Edu	ncation in Tropical and Coastal Areas: A Meta-Analysis	
D R Sawitri			
+ Open abstract	View article	PDF	
OPEN ACCESS			012051
	, ,	a Warna Dieng, Java Indonesia	
Tri Retnaningsih So	peprobowati, Sri Wido	do Agung Suedy and Hadiyanto	
+ Open abstract	View article	PDF	
OPEN ACCESS			012052
Improvement of Encapsulation Te	-	xidant Activities by Using Phycocyanin - Chitosan	
Meiny Suzery, Had	liyanto, Dian Majid, D	eny Setyawan and Heri Sutanto	
+ Open abstract	View article	PDF	
OPEN ACCESS			012053
Sea Water Intrusi	ion in Kaligawe Ser	marang Based on Resistivity Data	
Agus Setyawan, Na Ganap Febrika and	, ,	gnis Trihadini, Dhana Hastuti, Fitra Ramdhani, Fajar Waskito,	
•	View article	PDF	
OPEN ACCESS			012054
In Search for Sus	stainable Coastal M	anagement: A Case Study of Semarang, Indonesia	
Sudharto P Hadi			
+ Open abstract	View article	PDF	
OPEN ACCESS			012055
Effect of econom Countries	nic growth and envir	ronmental quality on tourism in Southeast Asian	
Firmansyah			
+ Open abstract	View article	PDF	
OPEN ACCESS			012056

Isolation And Partial Characterization Of Bacteria Activity Associated With Gorgonian *Euplexaura* sp. Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Pekalongan

Praba Ginandjar, Lintang Dian Saraswati, Opik Taufik, Nurjazuli and Bagoes Widjanarko

View article + Open abstract

7/26/2020

+ Open abstract

OPEN ACCESS

+ Open abstract

OPEN ACCESS

+ Open abstract

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Physical characteristics of phycocyanin from spirulina microcapsules using different coating materials with freeze drying method

E N Dewi, L Purnamayati and R A Kurniasih

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MJO (Madden-Julian Oscillation) Analysis of the Chlorophyll-a Distribution in Western Waters Bengkulu

Y D Haryanto, N Fitrianti, A Hartoko, S Anggoro and M Zainuri

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OPEN ACCESS 012062

Proximate Content of "Klekap" (Microphytobenthos and Their Associated Meiofauna) from Milk-Fish Pond

Widianingsih, Muhammad Zainuri, Sutrisno Anggoro and Hermin Pancasakti Kusumaningrum

View article 🔁 PDF + Open abstract

OPEN ACCESS 012063

10/13

Effects of Different Heat Processing on Fucoxanthin, Antioxidant Activity and Colour of Indonesian Brown Seaweeds

Eko Susanto, A Suhaeli Fahmi, Tri Winarni Agustini, Septian Rosyadi and Ayunda Dita Wardani + Open abstract View article 🔼 PDF **OPEN ACCESS** 012064 Determination and Radiocarbon Dating of Marine Mollusc Fossils in Ancient Sea Shelf of Central Java Indonesia S Aisyah, D Pringgenies, A Hartoko, J T S Sumantyo and H Matsuzaki + Open abstract View article 🔼 PDF **OPEN ACCESS** 012065 Sustainable Eco Coastal Development Through Corporate Social Responsibility (CSR) Program Arsi Rakhmanissazly, Yong Mursito Ardy and Abdullah + Open abstract View article 🄼 PDF **OPEN ACCESS** 012066 The effect of ENSO to the variability of sea surface height in western Pacific Ocean and eastern Indian Ocean and its connectivity to the Indonesia Throughflow (ITF) H A Rejeki, Munasik and Kunarso + Open abstract View article 🔼 PDF

OPEN ACCESS 012067

Antioxidant activity of three microalgae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana* clone Tahiti

Ita Widowati, Muhammad Zainuri, Hermien Pancasakti Kusumaningrum, Ragil Susilowati, Yann Hardivillier,

Vincent Leignel, Nathalie Bourgougnon and Jean-Luc Mouget

OPEN ACCESS 012068

Study on the effect of different concentration of *Spirulina platensis* paste added into dried noodle to its quality characteristics

T W Agustini, W F Ma'ruf, Widayat, B A Wibowo and Hadiyanto

OPEN ACCESS 012069

Study on Anticancer Activity of Extracts of Sponges Collected from Biak Water, Indonesia

A. Trianto, A. Ridhlo, D.W. Triningsih and J. Tanaka

+ Open abstract

View article

🔁 PDF

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		osan as Microbial Inhibitor Prawn Larvae Rearing	
	es, Muhammad Nur an	_	
+ Open abstract	View article	PDF	
OPEN ACCESS			012071
Production of Ch	itosan from <i>Amusii</i>	um sp Scallop Shell Waste	
Nur Rokhati, Titik	Istirokhatun, Dwi Titil	k Apriyanti and Heru Susanto	
+ Open abstract	View article	PDF	
OPEN ACCESS			012072
Achieving Resea	rch University: Inde	onesian Case	
Yos Johan Utama a	nd Ambariyanto		
+ Open abstract	View article	PDF	
JOURNAL LINK	XS .		
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Information for org	anizers		
Information for aut	hors		
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Low zinc serum levels and high blood lead levels among schoolage children in coastal area

Adriyan Pramono¹, Binar Panunggal^{1*}, M.Zen Rahfiludin², Fronthea Swastawati³

Abstract. The coverage of environmental lead toxicant was quiet wide. Lead exposure recently has been expected to be associated with zinc deficiency and blood indices disturbance. Emphasizing on children, which could absorb more than 50 % of lead that enters the body. Lead became the issue on the coastal area due to it has polluted the environment and waters as the source of fisheries products. This was a cross sectional study to determined nutritional status, blood lead levels, zinc serum levels, blood indices levels, fish intake among school children in coastal region of Semarang. This study was carried out on the school children aged between 8 and 12 years old in coastal region of Semarang. Nutritional status was figured out using anthropometry measurement. Blood lead and zinc serum levels were analyzed using the Atomic Absorbent Spectrophotometry (AAS) at a wavelength of 213.9 nm for zinc serum and 283.3 nm for blood lead. Blood indices was measured using auto blood hematology analyzer. Fish intake was assessed using 3nonconsecutive days 24-hours food recall. The children had high lead levels (median 34.86 $\mu g/dl$, range 11.46 - 58.86 $\mu g/dl$) compared to WHO cut off. Zinc serum levels was low (median 18.10 µg/dl, range 10.25 - 41.39 µg/dl) compared to the Joint WHO/UNICEF/IAEA/IZiNCG cut off. Approximately 26.4% of children were anemic. This study concluded that all school children had high blood lead levels, low zinc serum, and presented microcytic hypochromic anemia. This phenomenon should be considered as public health concern.

Keywords: blood lead, zinc serum, coastal region, microcytic hypochromic anemia, school children

1. Introduction

Zinc (Zn) is an essential trace element plays role as a cofactor of more than 100 metaloenzym, plays an important part in cell regeneration, metabolism, growth and immune function [1]. Zinc deficiency is associated with suboptimal growth, diarrhea, and decreased of immunity [2]. World Health

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Organization (WHO) estimates the prevalence of zinc deficiency in the world's population is ranged between 4 and 73%. Additionally, about 5 to 30% zinc deficiency has occurred in children and adolescents both in developed and developing countries [3]. Especially in the developing countries, harmful environmental exposure recently has been associated with the deficiency of Zn. Nutritional zinc deficiency can be caused from lead (Pb) exposure through dangerous cycle that increases lead absorption and increases Zn secretion consecutively [4]. Previous studies in animal experiments summarized that the conditions of marginal Zn deficiency, could increase blood lead levels in the presence of Pb exposure [5.6].

Among school children, chronic Pb exposure is correlated with nutritional deficiencies, anemia, impairments to physical growth, cognitive deficient and learning disorders [7 - 10]. Anemia is a major public health problem especially in developing countries. Additionally, WHO reported that of 25,4% anemia has been found in school children [11]. Anemia is a state of decreasing of erythrocytes and hemoglobin, whereas those condition impaired its function to carry oxygen through body tissues sufficiently [12,13]. Hemoglobin is composed of four globin chain polypeptide whereas in each chain contains a heme molecule containing Iron [14]. As well as iron, zinc also plays an important role in the formation of hemoglobin. In heme biosynthesis, δ -ALA dehydratase enzyme that catalyzes charge 2 δ -ALA into molecules that is highly dependent on zinc [15]. A study conducted in Atlanta gives us information that there is a significant correlation between serum zinc concentrations and hemoglobin levels since children who suffered iron deficiency anemia tend to be deficiency of zinc serum [16]. Normal zinc level has a consequence on erythrocytes life period, due to zinc contributes in protecting erythrocytes from oxidative stress and cell damage [17]. Increasing of blood lead levels could also interfered with erythropoiesis by inhibiting the synthesis of protoporphyrin and the absorption of iron (Fe) thus increasing the risk anemia. Furthermore, lead also affects the morphology of erythrocytes [18].

Nowadays, rapid growth of industrialization and its waste became environmental issues in coastal region of Indonesia. The industrial waste that was dumped in the coastal region induced the increasing level of lead pollutant in this area [19]. The coverage of environmental lead toxicant was quiet wide, including the exposure of fisheries products, public water source, and air pollution [20]. Lead exposure among children is an increasing problem globally, adversely affecting the quality of life among large numbers of children. The impact of lead exposure can affect all ages, whereas the children could absorbed more than 50 % of lead that enters the body, compared with adults who absorbed only 15-35 % [21]. The major vehicle of its exposure in the human body consecutively through the food consumption (65 %), the water consumption (20 %) or through the air (15 %) which were contaminated by lead [22]. In area with lead exposure, anemia and/or zinc deficiency might be occurred with or without clinical manifestation [20].

Lead exposure can not be seen as only a trivial environment and health problem. A study depicted even though given iron and zinc supplementation on lead-exposed children did not reduce blood lead levels [23]. Recently in Indonesia study about lead exposure among children mostly conducted in urban area, but not in coastal region. Especially among school children in Semarang city coastal region, the linking between blood lead levels with serum zinc level, anemia, and red blood cell indices has been not fully investigated.

2. Materials and Methods

2.1. Study population and site

A cross sectional study was conducted on an elementary school in Tambak lorok coastal region, Semarang, Central Java Indonesia. Tambak lorok is located less than 3 Kilometers from coastal java sea and near to harbor industrial region (Figure 1). About 80 subjects in this study were recruited using consecutive sampling method and met the inclusion criteria. During data collection, 8 subjects were dropped out due to did not agree to collect the blood. There were 72 children have completed data collection.

Inclusion criteria in this study: age between 8 to 12 years, and did not suffer from infectious illness (diarrhea, pneumonia, tuberculosis, and parasites) which were evidenced by looking at health records in primary health care. Demographic characteristics focused on parental educational level, parental occupation, source of water consumption, consumption of seafood products, age, and sex. Body weight was measured using calibrated digital body weight scale with accuracy 0.1 Kilogram. Body height was obtained using board stadiometer with accuracy 0.1 Centimeters. Median z score for weight for age, height for age, and BMI for age were performed to describe children nutritional status.

2.2. Biochemical collection and analysis

Biochemical data in this study consists of serum zinc level, red blood cells indices (Hb, Ht, mcv, mch, mchc), and blood lead level. About 10 mL venous blood has taken in the morning day before subjects have a breakfast using disposable plastic syringe and immediately transferred into three sterile-vacutainer. A potassium anticoagulant - EDTA (Ethylene Diamine Tetra Acetate) vacutainer was injected with 2 mL venous blood. This vacutainer was used to measure red blood cells (RBCs, in x 10⁶ /mL), hemoglobin (Hb, in g/dL), hematocrit (Ht, in g/dL) mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), and mean corpuscle hemoglobin concentration (MCHC). Blood indices were analyzed using automated Nihon Cohden Celltac E MEK-7222K hematology analyzer. Ferritin serum was analyzed using ELISA method.

The other two non-additives vacutainer were injected with 3 mL for serum zinc measurement and 5 mL for blood lead measurement. Approximately 3 mL whole blood in non-additive vacutainer was centrifuged, then the blood serum was prepared to zinc serum analysis. Substantially 5 mL whole blood was centrifuged and incorporated with APDC (Ammonium Pyrrolidine Dithiocarbamate) 2%. The incorporated solution was then re-centrifuged, allowed to stand for 5 minutes and mixed with 2 ml of butyl acetate. That mixed solution was centrifuged again until separation occurs. The reading of serum zinc level and blood lead level were operated using the Atomic Absorbent Spectrophotometry (AAS) at a wavelength of 213.9 nm for zinc serum and 283.3 nm for blood lead (Shimadzu AA6401F, Japan). Biochemical measurement was organized in the micronutrient and IDD Center laboratory, Diponegoro University, Indonesia.

Dietary assessment was conducted to determine fish intake among this population. The three-non consecutive days 24-hour food recall method was carried out to describe fish intake. In this study, local fish products have also been collected from this coastal area. Environmental assessment has been conducted on drinking water samples were collected using standard methods. Lead contents from fish products was measured using Spectrophotometry. Lead analysis of local fish products and drinking water samples were carried out in Center of Prevention on Industrial Pollution Laboratory, Ministry of Industrial, Republic Indonesia.

2.3. Statistical analysis

The SPSS for IBM version 19 and Microsoft excel software were performed for statistical analysis. All the study variables were tested for normality by the Kolmogorov-Smirnov test. The Mann Whitney test was operated to compare the blood lead levels and zinc serum levels between subjects with anemia and non anemia. The Correlation between blood lead level and serum zinc, hb, ht, RBC, mcv, mch, and ferritin were performed using Spearman rank's correlation analysis. Statistical significance was considered when p value < 0.05.

2.4. Ethics

The study protocol was approved by the Board of Medical Ethics on Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital with no. 534/EC/FK-RSDK/2015. The participants, who were recruited in this study, clearly informed about the purpose of investigations and expected outcomes. Informed consent was obtained and signed by the parents of the subjects before the study began.

3. Results

3.1. Demographic characteristics of respondents

The coastal region in this study was taken place at Tambak Lorok area, the northern area of Semarang, Central Java. This area borders along with java sea. The main harbor of Central Java province has been located in this coastal area. Industrial area that was built in this region may contributed to environmental and health problems issue. Figure 1 depicts environmental situation in this coastal region.

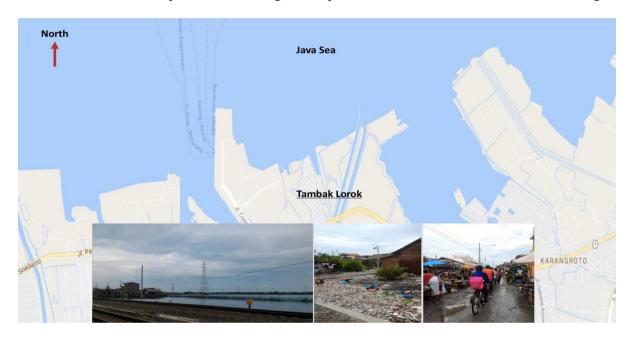


Figure 1. Environmental situation of Semarang coastal region (authors documented).

3.2. Biochemical data analysis

Table 1 shows values of haemoglobin, hematocrit, red blood indices, zinc serum levels, blood lead levels among all children. All subjects had blood lead levels \geq 10 (µg/dl), low zinc serum levels (< 65 µg/dl), and median of ferritin describes < 15 ng/ml.

Table 1. Mean, SD, median, minimum and maximum values of blood indices, zinc serum, blood lead levels, and ferritin among subjects (N=72)

Biochemical parameters	Median	Range (min – max)
Haemoglobin (g/dl)	12.85	10,00 - 15,6
Hematocrit (g/dl)	35.40	28,70 - 44,70
RBC ($\times 10^6 \text{ mm}^3$)	4.82	4.21 - 6.00
$MCV(\mu^3)$	74.45	52.10 - 81.05
MCH	27.00	18.10 - 29.70
MCHC	36.30	34.10 - 38.30
Ferritin (ng/ml)	13.56	2.28 - 44.68
Zinc serum (µg/dl)	18.10	10,25 - 41,39
Blood Lead Level (µg/dl)	34.86	11.46 - 58.86

This study was carried out to 72 children with age range from 9 to 13 years with a mean value of 10.16 ± 1.02 years. Approximately 66.7% of children's age were categorized between 9 and 10 years old.

Surprisingly, about 90.3% of children were stunting, consists of mild stunting 34.7%, moderate stunting 45.8%, and severe stunting 9.7%. We can see from table 1 about 26.4% children were anemic. Father's occupation of subjects largely as fisherman (59.7%). Both father and mother of children had literate, mostly graduated from Senior High School (father 51.4%, mother 45.8%) (Table 2).

Table 2. Characteristics of school age children (N=72)

Characteristics	N (%)
Sex	11 (70)
Male	39 (54.2)
Female	33 (45.8)
Age	, ,
9 – 10	48 (66.7)
11 - 12	24 (33.3)
Height for Age Nutritional Status	
Normal	7 (9.7)
Mild Stunting	25 (34.7)
Moderate Stunting	33 (45.8)
Severe Stunting	7 (9.7)
Anemia Status	
Anemic	19 (26.4)
Non Anemic	53 (73.6)
Father literacy	
Graduated elementary school	13 (18.1)
Graduated junior high school	22 (30.6)
Graduated senior high school	37 (51.4)
Mother literacy	
Graduated elementary school	16 (22.2)
Graduated junior high school	23 (31.9)
Graduated senior high school	33 (45.8)
Father's occupation	
Salesman	5 (6.9)
Fisherman	43 (59.7)
Labor	24 (33.3)
Mother's occupation	
Household	36 (50.0)
Salesman	2 (2.8)
Labor	34 (47.2)

Comparison between mean values of different hematological parameters, blood lead levels, zinc serum levels and serum ferritin in anemic and non anemic were figured out in this study (Table 3). Regarding the hematological parameters, nearly all values were significantly lower among the anemic than the non-anemic group except for blood lead levels, which showed a highly significant elevation among the anemic group. However, according to World Health Organization (WHO) cut off for blood lead levels, all children in this study had a blood lead $\geq \! 10\,\mu g/dl$ (high blood lead level group {HBLLs}). As for the RBC count, ferritin, and zinc serum, no statistically significant difference was detected between the groups.

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Table 3. Comparison between mean values of different hematological parameters, zinc serum, blood lead levels, and serum level of ferritin in anemic and non-anemic groups.

Biochemical	Anemic Group (N=19)	Non Anemic Group (N=53)	Test of
<u>parameters</u>			Significance
RBC ($\times 10^6 \text{ mm}^3$)	4.82 <u>+</u> 0.49	4.88 ± 0.33	t = -0.56
Hb (g/dl)	11.65 ± 0.62	$13.26 \pm 0.77*$	p = 0.58 t = -9.03 p = 0.000
Hct	32.66 <u>+</u> 1.44	36.47 <u>+</u> 1.88*	t = -9.05 p = 0.000
$MCV(\mu^3)$	68.43 ± 7.50	$74.89 \pm 3.74*$	$ \begin{array}{r} p = 0.000 \\ t = -4.84 \\ p = 0.000 \end{array} $
MCH	24.45 ± 3.05	27.26 ± 1.65**	t = -5.01 p = 0.000
МСНС	35.66 ± 0.92	36.39 ± 0.75 *	t = -3.09
Ferritin (ng/ml)	15.59 <u>+</u> 8.39	15.36 <u>+</u> 8.58	p = 0.005 t = 0.10 p = 0.92
Zinc serum (µg/dl)	20.13 <u>+</u> 9.64	20.44 <u>+</u> 8.31	t = -0.12 p = 0.90
Blood lead level (μg/dl)	41.95 ± 10.61	32.03 ± 10.33**	p = 0.90 t = 3.52 p = 0.001

^{*}independent t-test were performed (significant if p<0.05)

Table 4 reveals the correlation between the different red blood indices, serum zinc levels and the blood levels of lead. Blood lead levels had a significant negative correlation with haemoglobin, hematocrit, and zinc serum (r = -0.330, r = -0.328, respectively p<0.005 and r = -0.265, p<0.05).

Table 4. Correlation of different hematological parameters, serum iron and ferritin levels in relation to blood lead, copper and zinc.

Hematological parameters	Blood lead levels r-value (p-value)	Zinc serum levels r-value (p-value)
RBC (×10 ⁶ mm ³)	-0.066 (0.585)	-0.128 (0.285)
Hb (g/dl)	-0.330 (0.005)	0.119 (0.319)
Hct	-0.328 (0.005)	0.101 (0.396)
$MCV(\mu^3)$	-0.124 (0.298)	0.130 (0.276)
MCH	-0.109 (0.363)	0.148 (0.214)
MCHC	-0.106 (0.375)	0.144 (0.227)
Ferritin (ng/ml)	0.178 (0.135)	0.075 (0.529)
Zinc serum levels	-0.265 (0.025)	-
Blood lead levels	-	-0.265 (0.025)

3.3. Environmental assessment

Environmental assessment has been conducted on drinking water samples and dietary fish intake. Local fish products were collected based on the information from dietary fish intake interview. Lead content of drinking water and local fish products can be seen in Table 5.

^{**}Mann Whitney test were performed (significant if p<0.05)

Table 5. Fish intake (g per day) and lead levels of drink water and local fish products

Parameters	Mean	Median	Range (min – max)
Fish intake (g/day)		40	10,0 – 128
Lead from drinking water		0	0
(μg/dl)			
Lead from some type of fish			
(mg/Kg)			
- Green mussels (Pena viridis)	1.13		
- Scallop shell (Anadara granosa)	0.76		
- Kipper fish (Scatophagidae)	0.18		
- Snapper fish (Lates calcalifer)	0.13		
- Mullets fish (Mugil cephalus)	0.18		

4. Discussion

All school children in this study (100%) had BLL $\geq 10~\mu g/dl$ and zinc serum levels < 65 $\mu g/dl$, similar to a study done by Hegazy et al [7]. who also reported about more than a half subjects (63.3%) had BLL $\geq 10~\mu g/dl$. However the current study is similar to the results obtained for children population in India [24]. The cut off value of 10 $\mu g/dl$ defined by the WHO guideline for Childhood Lead Poisoning as a limit for an elevated blood lead level primarily is based on neurological toxicity and has wide range of toxicity including neurobehavioral impairment [21]. Recently, even though blood lead levels (BLL) less than 10 $\mu g/dl$ is considered safe, a study confirmed that BLL < 10 $\mu g/dl$ has associated with cognitive deficits [25]. Thus our data showed all children had low zinc serum levels (< 65 $\mu g/dl$). The Joint WHO/UNICEF/IAEA/IZiNCG asserted zinc serum levels < 65 $\mu g/dl$ has been recognized as serious public health problems [26].

Schwartz et al [28] reported that children living near lead smelters in the US of Idaho, had blood lead levels approximately 25 μ g/dl and were correlated with anaemia. In addition, Jain et al [29]. reported that children with BLL>10 μ g/dl had 1.7 times risk to be moderate anaemia, in contrast with our finding showed that 73.6% of children had normal haemoglobin. Froom et al [30]. suggested that haemoglobin level did not correlate well with BLLs. However, our finding described that Hb, Hct, MCV, MCH and MCHC values of children with anaemia lower in comparison to anemic group. Moreover high BLLs has negatively associated with lower zinc serum and some blood indices levels. Lead absorption occurs predominantly in the duodenum and jejunum. The process of lead entry into the body may carry on through food, drink or by air. Approximately 90 % of lead absorbed by the blood binds to red blood cells [27]. The children absorbed up to 50% of lead from food and/or drink through the gastrointestinal tract and will be included in the metabolic processes of the body. Those lower red blood indices is similar with hypochromic microcytic anaemia which can be caused by blood lead toxicity [7]. Lead causes anaemia by impairing heme synthesis and increasing the rate of red blood cell destruction [31]. Although a causal pathway cannot be determined, yet the study findings clearly demonstrate the differences of BLLs between anaemia status.

In the present study zinc serum level of the anemic group is insignificantly different than non-anemic group. Lead (Pb) has the same valiancy number (Pb2+) as zinc (Zn2+) and iron (Fe2+), whereas on the cellular transport by Dimetal Transporter-1 (DMT-1) competition may be occurred [32]. Lead (Pb2+) will always be dominant to carry on by DMT-1 because of its density higher than Zn2+ and Fe2+

[27]. Lead (Pb) inhibits δ -ALA dehydratase enzyme that catalyzes δ -ALA into molecules, which is highly dependent to zinc on haemoglobin synthesis [15].

In the current study high BLLs among school children may be due to air pollution, sewage contamination, and probably from food consumption which were contaminated with Lead (Pb). Based on dietary interview, children consume fish regularly (range between 10 g/day and 128 g/day). Thus based on lead measurement content on some fish products showed variety lead content (mg/Kg fish products), green mussels (Pena viridis) had the highest content of lead (1.13mg/Kg). Green mussels (Pena viridis) has been vended on street food vendor near by school building. However a causal pathway between some fish products intake and blood lead levels cannot be firmed only from this data. A large epidemiology study on general northern coastal communities should remain a concern because of the nature of accumulation.

In developing countries such as Indonesia, control of lead contamination is much slower and the negative health effects getting more sporadic. The present work revealed that lead contamination should be considered as public health concern in northern coastal population of Semarang, Central Java, Indonesia.

5. Conclusion

In the present study, all subjects has high blood lead levels (BLLs) and low zinc serum. Blood lead levels (BLLs) were negatively associated with the hematological parameters and zinc serum levels. Lead (Pb) was not presented in drinking water, but Pb was discovered in some fish products that may be regularly consume by the children. Lead exposure could be controlled and strides should be taken to reduce zinc deficiency and anaemia among children population at coastal region.

6. References

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