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Superoxide dismutase (SOD) activity of *Ceriops zippeliana* in Segara Anakan Cilacap (Indonesia) under heavy metal accumulation

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Abstract. *Sukmarani D, Proklamasiningsih E, Susanto AH, Ardli ER, Sudiana E, Yani E. 2021. Superoxide dismutase (SOD) activity of* Ceriops zippeliana *Blume in Segara Anakan Cilacap (Indonesia) under heavy metal accumulation. Biodiversitas 22: 5627-5635.* *Ceriops zippeliana* Blume is a true mangrove species that occupy habitats exposed to environmental stress, such as salinity and heavy metal contamination, which is the case in Segara Anakan Cilacap, Central Java. Both salinity and heavy metal stress can lead to the production of reactive oxygen species (ROS), which can be detrimental to plants if they are present in excess. Plants have defense mechanisms to prevent excessive ROS, one of which is by the use of superoxide dismutase (SOD) enzyme. This study aims to explain the accumulation of heavy metals (Pb, Cd, Cu, and Zn) in *C. zippeliana* as well as the translocation of the metals. In addition, the correlation between heavy metal concentrations and SOD activity in *C. zippeliana* is reported. Plant samples were randomly collected from Segara Anakan, Cilacap. The analysis was conducted using the bio-concentration factor (BCF), translocation factor (TF), and linear regression in the SPSS program. It was found that Pb, Cu, and Zn levels in Segara Anakan Cilacap sediments were still within acceptable limits, while Cd level was moderately polluting. Metal accumulation was higher in the branches of *C. zippeliana* compared to those in roots and leaves. The SOD activity of *C. zippeliana* in Segara Anakan is seemingly not related to metal contents in plant parts.

Keywords: Bio-concentration factor, *Ceriops zippeliana*, heavy metal accumulation, SOD activity, translocation factor

INTRODUCTION

Ceriops zippeliana Blume is one of the two *Ceriops* species (Rhizophoraceae) that occurred in Indonesian mangrove forests, while the other one is *C. tagal*. According to the IUCN Red List, *C. zippeliana* is categorized as Least Concern with decreasing population trend (IUCN 2008). As a member of Rhizophoraceae, which is the most common mangrove family in Indonesia, one area of *C. zippeliana* geographical distribution is Segara Anakan Cilacap, Central Java. This is an estuary or delta located at the mouth of main rivers in Central Java south coast heading to the Indian Ocean. Despite being blocked by Nusakambangan Island from the open ocean, mangroves in Segara Anakan receive the influence of the Indian Ocean currents. *C. zippeliana* grows in the eastern part of Segara Anakan Cilacap. The mangrove vegetation in this area is pressured by land conversion as well as pollution caused by an oil refinery industry (Supriatna et al. 2018; Sih Piranti et al. 2019; Setyaningrum et al. 2020).

As a true mangrove species, *C. zippeliana* has extensive morphological, anatomical, physiological and molecular adaptations, allowing growth and development in environments with significant environmental stress, such as salinity and heavy metal (Rodriguez et al. 2012; Srikanth et al. 2016; Surya and Harti 2017). This mechanism can be explained by the buildup of reactive oxygen species (ROS) as one of the metabolic alterations that occur when plants are exposed to salt and heavy metal stress (Hasanuzzaman et al. 2020). Plants produce ROS as a consequence of regular cellular metabolism. However, when there is a disruption in the environment (e.g. increased salinity and pollution), the equilibrium between production and elimination is disturbed. When present in excess, ROS rapidly inactivate enzymes, damage vital cellular organelles in plants, and destroy membranes by inducing the degradation of pigments, proteins, lipids, and nucleic acids, ultimately resulting in cell death (Karuppanapandian et al. 2011). Raja et al. (2017) found that ROS can disturb normal metabolism by causing oxidative damage to lipids, proteins, and nucleic acids, as well as impairing membrane function (Hasanuzzaman et al. 2020).

Not all ROS are free radicals. Some ROS are radicals, such as superoxide anion (O_2^-), hydroperoxyl radical (HO_2), alkoxy radical (RO) and hydroxyl radicals (OH^-), while others are non-radicals, such as hydrogen peroxide (H_2O_2) and singlet oxygen (O_2). Superoxide radicals are extremely harmful when combined with hydrogen peroxide (H_2O_2) because they form hydroxyl radicals (OH^-) (Hasanuzzaman et al. 2020).

Plants have defense mechanisms to deal with environmental stress. Activating antioxidant enzymes is one of the antioxidant defense strategies in plants. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase are some of the enzymes involved. SOD catalyzes the dismutation of superoxide radicals (O_2^-) into H_2O_2 and O_2; catalase degrades H_2O_2 into water and oxygen; and glutathione peroxidase catalyzes the reduction of H_2O_2 to H_2O using reduced glutathione (GSH) and oxidized glutathione (GSSG) as cofactors (Hasanuzzaman et al. 2017). SOD is considered to be at the forefront of overcoming oxidative stress due to its position as the first enzyme to catalyze superoxide radicals (O^{2-}) (Ighodaro and Akinloye 2018).

There has been no investigation on heavy metal bioaccumulation and its correlation with SOD activity in *C. zippeliana* from Segara Anakan Cilacap so far. As a result, such studies must be conducted to provide information on the long-term conservation of *C. zippeliana* as well as the management of estuaries and coastal areas. This study aims to elucidate the accumulation of heavy metal Pb, Cd, Cu, and Zn in *C. zippeliana,* as well as the translocation of the metals in *C. zippeliana*. The correlation between heavy metal concentrations and SOD activity in *C. zippeliana* is also investigated.

MATERIALS AND METHODS

Study area and period

Samples were collected randomly from the eastern section of Segara Anakan lagoon, Cilacap District, Central Java, Indonesia at geographical position of 7°68'91" S 108°99'35" E. They were collected at three different sites along the estuary, from the outside to the inside, across the oil refinery industries (Figure 1). The sampling was accomplished in July 2020.

Procedures

Sample collection and identification

Sediments as well as roots, branches, and leaves of *C. zippeliana* were taken randomly from three different sites in the canal. Every sample was weighed up to 500 g. The samples were placed in zip lock bags, labeled, and stored in an icebox. The *Flora of Java* by Backer and Bakhuizen van Den Brink (1963) determination book was used for identification.

Heavy metal content analysis

Analyzing the metal contents of Cu, Zn, Pb, and Cd in sediments and plant parts began with drying the sample in an oven for 6 hours at 80°C for plants and 105°C for sediments. The aqua regia method (HNO_3: HCl = 1: 3) was used to digest 5 g of the sample, which was heated at 100°C for 8 hours until the volume reached 1 mL. Before heating, hydrofluoric acid (HF) was added to the sediment samples. The sample was then cooled to room temperature and transferred to a 100 mL volumetric flask, where aquabidest were added to the mark—then filtered using 0.40 μm pore filter paper. AAS (Atomic Absorption Spectroscopy) was used to determine metal concentration (Takarina and Pin 2017). The recovery rates of heavy metals in CRM are listed in Table 1.

Table 1. Recovery rates of heavy metal concentrations measured in certified reference material and standard deviation

Element	Certified value GSD-10 (mg/kg)	Measured value (N = 3) (mg/kg)	Recovery rate (%)
Cu	22.6±0.6	20.33±4.72	90
Zn	46±1.3	32.22±0.20	70
Pb	27±1	27.38±0.86	101
Cd	1.12±0.05	1.73±0.04	154

Figure 1. Map of the sampling sites in Segara Anakan lagoon, Cilacap District, Central Java, Indonesia

Superoxide dismutase (SOD) activity assay

The SOD activity assay started with extraction of roots, leaves, and branches that have been dried in an oven at 70°C for 48 hours before being mashed. Extraction was performed with a 96% ethanol solvent using the maceration process. SOD activity assay was performed according to the RANSOD Manual by Randox Laboratories, with the following modifications: 20 μL of sample was added to a combination of 1,000 μL R1 (1 bottle of mixed substrate R1a with 20 μL Buffer R1b) and 100 μL R2 (1 vial of xanthine oxidase dissolved with 10 mL of distilled water). The absorbance of the reaction mixture was measured at λ 520 nm (Randox Laboratories 2009).

Data analysis

Bio-concentration factor (BCF)

Bio-concentration factor (BCF) can be used to determine the value of metal bioaccumulation (Pb, Cd, Cu, and Zn) in plant sections of *C. zippeliana*, either by active or passive accumulation. BCF is the ratio of individual metal concentration in the tissue ($C_{tissues}$) to that in the sediment ($C_{sediment}$) (Takarina and Pin 2017).

$$BCF = C_{tissues}/C_{sediment}$$

Translocation factor (TF)

The metal translocation process among parts of the *C. zippeliana* plant was calculated using the translocation factor (TF), which is the ratio of metal concentration between two plant parts (Takarina and Pin, 2017):

$$TF_{root\ to\ branch}=C_{branch}/C_{root}$$
$$TF_{root\ to\ leaves}=C_{leaves}/C_{root}$$
$$TF_{branch\ to\ leaves}=C_{leaves}/C_{branch}$$

Superoxide dismutase (SOD) activity

Pearson correlation in the SPSS program was utilized to establish the correlation between metal concentration and SOD activity (Bujang and Baharum 2016).

RESULTS AND DISCUSSION

Heavy metal concentrations in sediments

Lead (Pb) concentration in the Segara Anakan sediments was found ranging from 26.53 to 28.24 mg/kg (Table 2) with an average of 27.38 mg/kg (Table 3). These are still within the tolerable levels compared to those in other mangrove locations (Table 2). In natural environment, it is available at 70 mg/kg or lower, and could be toxic to plants when presents at 100 to 500 mg/kg (Kabata-Pendias and Pendias 2000). Meanwhile, the Pb concentration is also still much below the standard for agricultural soils in some countries, i.e. 80 mg/kg (EPMC, China), 200 mg/kg (CME, Canada), 200 mg/kg (TMS Tanzania), 200 mg/kg (USEPA, USA), 300 mg/kg (EPAA, Australia), 530 mg/kg (EEA, Netherlands), 1,000 mg/kg (EEA Germany) (He et al. 2015), and 4,000 mg/kg (NOAA) (Buchman 1999).

Pb is a toxic element that causes various physiological consequences, including decreased seed germination, root elongation, lower biomass, inhibition of chlorophyll biosynthesis, mineral feeding and enzymatic responses. The severity of these effects depends on the length of exposure, the stage of plant development, the organ understudy, and the Pb concentration involved. Pb enters plants mostly through roots, not only the apoplast route or calcium ion channels (Pourrut et al. 2011). On the other hand, Pb enters plants in little amounts through leaves (Ghosh and Roy 2019). The sampling site in Segara Anakan Cilacap was located less than 1 km from an oil-refinery area with various pollutants, including air pollution. As one of the pollutants from the oil refinery industry, Pb can also enter the leaves through the air, along with other heavy metals (De Agostini et al. 2020), but it is not used in plant physiological processes. This could explain why the concentration of Pb in the leaves is higher than that of Zn or other metals.

The concentration of cadmium (Cd) in the Segara Anakan sediments in this study ranged from 1.69 to 1.77 mg/kg (Table 2) with an average of 1.73 mg/kg (Table 3), which is higher than those of natural levels. Nevertheless, the Cd concentrations obtained are not excessively high or moderate compared to those in other places (Table 2). Cd values of 0.1-1 mg/kg are found naturally in soil (Smolders and Mertens 2013). Standards for Cd in agricultural soils from numerous countries are 0.11 mg/kg (US EPA USA), 0.3-0.6 mg/kg (EPMC China), 1 mg/kg (TMS Tanzania), 3 mg/kg (EPAA Australia), 3 mg/kg (CME Canada), 13 mg/kg (EEA Netherlands), 50 mg/kg (EEA Germany) (He et al. 2015), and 100-300 mg/kg (NOAA) (Buchman 1999). This means that Cd concentration in the Segara Anakan sediments is higher than natural values and exceeds American, Chinese, and Tanzanian standards, but still below the standard of others.

Cd is a non-essential element that has deleterious impact on plant growth and development. Because of the generation of reactive oxygen species (ROS), Cd can cause metabolic problems in plants by inhibiting nitrogen uptake and transport, reducing plant growth, lowering chlorophyll and carotenoid content, and inhibiting photosynthesis. Cd enters plant tissues through the soil and water, along with other nutrients, which are absorbed through roots, transported through xylem, and stored in various tissues (Ghosh and Roy 2019). Cd is generally accumulated in vacuoles or cell walls. This accumulation was identified as one of the Cd detoxification and tolerance mechanisms (Uddin et al. 2021). It restricts and limits the free circulation of Cd ions in the cytosol. Like other metal ions, Cd can be taken from the air, deposited on the leaf surface, and subsequently absorbed into plant tissues (Ghosh and Roy 2019).

Copper (Cu) concentrations in mangrove sediments in Segara Anakan ranged from 15.61 to 25.06 mg/kg (Table 2) with an average of 20.33 mg/kg (Table 3), which are still moderate when compared to those in some other places (Table 2). Cu standards in agricultural soils are 100 mg/kg (EPAA Australia), 150 mg/kg (CME Canada), 150-300 mg/kg (EPMC China), 190 mg/kg (EEA Netherlands), 200 mg/kg (EEA Germany), 200 mg/kg (TMS Tanzania), 270 mg/kg (US EPA USA) (He et al. 2015), and 10,000-25,000 mg/kg (NOAA) (Buchman 1999). Thus, it could be said that Cu levels in Segara Anakan sediments are still below the standards for agricultural soils in the countries.

Since required in enzyme systems related to photosystem II electron transport, mitochondrial and chloroplast reactions, carbohydrate metabolism, cell wall lignification, and protein synthesis, Cu is an essential element for plant growth. This metal, in particular, serves as a cofactor for metalloenzymes (Kumar et al. 2021). Cu can be toxic to plant growth if the concentration level in the sediment exceeds 800 mg/kg (Marques et al. 2018). This is about 40 times higher than those observed in Segara Anakan sediments (Table 3).

Compared to the other metals, the concentration of Zinc (Zn) in the sediment, roots, and branches of *C. zippeliana* in Segara Anakan was found highest. This is as Marschner (2011), who claims that Zn is the second most prevalent transition metal in living organisms after iron (Fe) (Balafrej et al. 2020). Zn concentrations in Segara Anakan sediments ranged from 32.03 to 32.43 mg/kg (Table 2) with an average of 32.23 mg/kg (Table 3). This concentration has not exceeded its normal level, and it is also quite moderate compared to other locations (Table 2). Natural levels in soils and rocks are typically between 10 and 300 mg/kg, while those in rivers are often less than 0.2 mg/kg (Noulas et al. 2018). Zn standards for agricultural soils in some countries are 150 mg/kg (TMS Tanzania), 200 mg/kg (EPAA Australia), 200-300 mg/kg (EPMC China), 500 mg/kg (CME Canada), 600 mg/kg (EEA Germany), 720 mg/kg (EEA Netherlands), 1,100 mg/kg (US EPA USA) (He et al. 2015), and 7,000-38,000 mg/kg (NOAA) (Buchman 1999). This indicates that Zn levels in Segara Anakan sediments are less than the standards.

Zn is found in soil due to the pedogenetic process of washing the source rock (Wuana and Okieimen 2011). This metal influences cell division and differentiation, as well as plant development, reproduction, and cell signaling which includes DNA replication, transcription, and protein synthesis. Many enzymes, such as carbonic anhydrase, carboxypeptidase, and Zn-superoxide dismutase, require Zn as a cofactor (Balafrej et al. 2020).

According to NOAA, the sequence of metal concentrations from high to low is Cu/Zn>Pb>Cd, implying that Cu may be greater than Zn, or that Zn may be greater than Cu. In addition, Pb concentration comes after Cu or Zn, and Cd comes last (Buchman 1999). Sediment metal concentrations in Segara Anakan were Zn>Pb>Cu>Cd. The Pb concentration is still below the international standard set by various countries and the NOAA, as previously stated. Even so, because the Pb concentration was higher than the Cu concentration, it can be said that Pb in Segara Anakan was relatively high.

Heavy metal concentrations in plant parts compared to sediments

It can be seen from the analysis of sediment samples from the three different sites and a total of nine samples of *C. zippeliana* plant parts that a relatively similar distribution of elements in sediments, roots, and branches were observed, i.e. Zn>Pb>Cu>Cd. However, this was not the case with leaves, where Pb>Zn>Cu>Cd (Table 3). The average concentration level of individual metal in the plant parts of *C. zippeliana* showed a relatively similar pattern, in which branches had the highest metal concentrations followed sequentially by roots and leaves. This demonstrates that roots, as opposed to branches, have a stronger potential to absorb metals rather than accumulate them (Takarina and Pin 2017). On the other hand, the branch tissue can store more metals, including non-essential metals (Arumugam et al. 2018). This could be related to a long-term accumulation of metals in the branches (Marchiol et al. 2004). Roots are more susceptible to metals than shoots are, because roots are the first organs to come into touch with metals (in the soil), and they accumulate metals in higher proportions than shoots do (Tiryakioglu et al. 2006; Hilmi et al. 2017).

Toxicity and even the usefulness of an element for plants are both subjective. Plants can demonstrate a phytotoxic response or tolerance with the buildup of metals in their bodies, as indicated by Mishra et al. (2006), depending on their demands, endurance, and environment. Phytotoxic responses such as chlorosis, necrosis, wilting, decreased growth, lower biomass (Kumar et al. 2016), stunting, and decreased crop yields, have all been observed. Meanwhile, the tolerance response can be indicated by enzyme activity (Hasanuzzaman et al. 2020) or detoxifying mechanisms, such as selective metal absorption, excretion, synthesis of metal compounds with specific ligands, and metal compartmentalization (Pourrut et al. 2011).

Table 2. Heavy metal concentrations in sediment samples from eastern part of Segara Anakan compared to other locations

Locations	N	Heavy metal concentrations (mg/kg)				References
		Pb	Cd	Cu	Zn	
Segara Anakan Cilacap	3	26.53-28.24	1.69-1.77	15.61-25.06	32.03-32.43	This study
Segara Anakan Cilacap	34	-	-	16-84	39-480	(Syakti et al. 2015)
Segara Anakan Cilacap	6	3.96-21.99	0.43-2.21	-	-	(Hidayati et al. 2014)
Jakarta Bay	4	<LOD-357	<LOD-13	<LOD-515	-	(Siregar et al. 2016)
Coast of Red Sea	15	13.5-230	ND-5.48	45.5-280	3.95-275	(Usman et al. 2013)
Hainan Island, Cina	12	11-19	0.06-0.13	9-18	32-57	(Qiu et al. 2011)
Kathmandu, Nepal	60	20.96-31.81	0.23-0.30	25.39-27.91	35.15-59.70	(Yan et al. 2012)

Note: the values are based on the range from minimum to maximum concentration from each study. (N: number of samples; -: not measured)

Table 3. Heavy metal concentrations in sediments and *Ceriops zippeliana* samples from eastern part of Segara Anakan

Source	N	Mean of heavy metal concentration ± stdev (mg/kg)			
		Pb	Cd	Cu	Zn
Sediment	3	27.38±0.86	1.73±0.04	20.33±4.72	32.22±0.20
Root	3	16.68±9.87	0.92±0.84	7.82±2.10	25.48±12.11
Branch	3	29.64±0.50	2.11±0.13	26.07±32.23	41.94±38.80
Leaves	3	13.43±0.59	0.71±0.07	2.33±0.07	6.78±3.38

Bio-concentration factor (BCF)

Based on the average value of BCF, Zn>Pb>Cd>Cu sequence of concentration levels in the roots with an average value of less than 1 was observed. Different sequence of Zn>Cd>Cu>Pb with an average value of more than 1 was found in the branches, while Pb>Cd>Zn>Cu with an average value of less than 1 was obtained in the leaves (Table 4). The Bioconcentration factor value is divided into 3 (three) categories; with a BCF value >1 is classified as an accumulator, BCF value = 1 can be categorized as an indicator, while BCF value <1 is classified as an excluder (Mastaller 1996 *in* Isroni et al. 2020). Each species has a different ability to absorb heavy metals from its environment. Several other mangrove species have a high capacity to absorb heavy metals from their environment. Even *Xylocarpus granatum*, *Sonneratia alba*, *Rhizophora apiculata*, *Bruguiera gymnorrhiza*, *B. parviflora*, *C. tagal,* and *Lumnitzera racemosa* can have a BCF value of more than 2 (Analuddin et al. 2017). In addition, the older tissue can store more metal, and the BCF value can be greater (Mejías et al. 2013). In this study, the BCF values indicate the ability of *C. zippeliana* to store metals absorbed from the environment in its plant tissues. It is likely that in *C. zippeliana* the ability to store metals in branches is higher than that in roots and leaves.

Table 4. Bio-concentration factor (BCF) of heavy metals in *Ceriops zippeliana* samples from eastern part of Segara Anakan

Plant parts		Pb	Cd	Cu	Zn
Root	Min	0.25	0.06	0.33	0.27
	Max	1.07	1.18	0.50	1.12
	Mean	0.60	0.53	0.42	0.79
Branch	Min	1.03	1.09	0.15	0.39
	Max	1.12	1.34	2.86	2.98
	Mean	1.07	1.21	1.09	1.30
Leaves	Min	0.48	0.37	0.10	0.07
	Max	0.50	0.45	0.15	0.31
	Mean	0.49	0.41	0.13	0.21

Translocation factor (TF)

Translocation factor (TF) is used to analyze the movement of elements from roots to shoots and to evaluate the phytoextraction capability of plants by comparing metal concentrations in leaves, branches, and roots (Marchiol et al. 2004). Figure 2 shows that the highest TF value was found from roots to branches with an average of more than 1, where Cu>Cd>Pb>Zn was observed. In contrast, the TF values from roots to leaves and from branches to leaves with an average of less than 1 respectively were obtained. This indicates that metal translocation from roots to stems is better than that from roots to leaves, where the speed of translocation is affected by the transport network capillary system (Takarina and Pin 2017). It is also revealed that metal accumulates continuously in branches throughout time and that branches have a higher phytoextraction potential than those of roots and leaves (Marchiol et al. 2004).

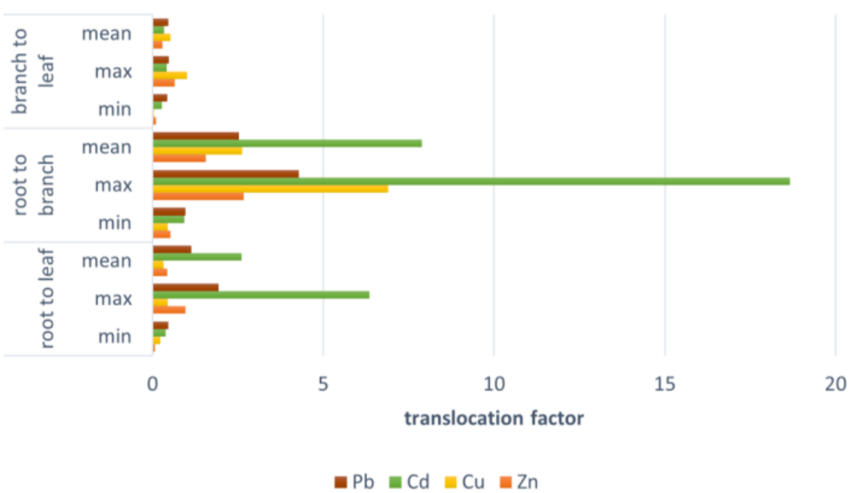

Figure 2. Diagram of each metal's translocation factors (TF) in plant sections of *Ceriops zippeliana*

Zn was detected at the highest concentration in the roots and branches of *C. zippeliana*. The high Zn concentration in the roots and branches may be due to its high concentration in the sediments (Thanh-Nho et al. 2018). Each metal concentration was found higher in the roots than in the leaves. This may occur because roots are the organs that interact first with various elements and metals in sediments. Moreover, the highest metal concentration in the branches suggests that *C. zippeliana* could accumulate metal in tissues (Jadia and Fulekar 2008).

The concentration of Pb in the leaves was found higher than the other three metals. This can be the case because Pb enters the plant through the leaves from the air, especially since the sample was taken from near an oil refinery, contributing to Pb contamination through the air. On the other hand, Cu can be bound to organic debris, reducing its availability in leaves (Lacerda et al. 1993). Cu and Zn concentrations in leaves are thought to be lower than that of Pb because they are widely used in biological metabolic activities (Lacerda et al. 1993; Arumugam et al. 2018).

C. zippeliana was shown to have phytoremediation capability by accumulating most metals in its branches as can be seen from BCF and TF values obtained in this study. This is in accordance with Jadia and Fulekar (2008), suggesting that tolerance to heavy metal, high metal accumulation ability, abundant root system, and high bioaccumulation factor are some of the characteristics for plants that can be applied for phytoremediation. Lacerda et al. (1993) also noted that BCF might be used to determine how well plants adapt to their environments.

Correlation between metal concentration and SOD activity in *Ceriops zippeliana*

It was found that branches showed the highest SOD enzyme activity (155.57±15.66 U/ml), followed by leaves (104.2±30.07 U/ml) and the roots (98.7±33.90 U/ml). The significance value of the p-value for each element was 0.064 for Pb; 0.070 for Cd; 0.967 for Cu; and 0.0992 for Zn when it was correlated to metal concentration in individual part of the plant using Pearson correlation. All the four metal elements showed a p-value greater than 0.05, implying no significant relationship between metal concentrations in plant parts and SOD enzyme activity overall. Several previous studies have also shown that enzyme activity can increase or decrease in response to metal treatment (Tiryakioglu et al. 2006; Hu et al. 2007).

The activity of the SOD can decrease or increase depending on the amount of Pb present. In this case, individual plant has also unique response. SOD activity can increase and decrease in aquatic plants *Potamogeton crispus* due to differences in Pb treatment (Hu et al. 2007). When compared to control plants, SOD activity decreased at 10-20 mg/L Pb concentrations but increased again at 50 mg/L Pb concentrations. Lethal Pb concentrations in *P. crispus* are estimated to be between 10 and 15 mg/L. Mishra et al. (2006) reported different responses in the aquatic plant *Ceratophyllum*, showing that *Ceratophyllum* could show an increase in SOD as a positive tolerance response at moderate Pb concentrations (1-25 μm) and that SOD activity decreased with increasing duration and Pb concentration (100 μm) (Hu et al. 2007). Thus, it can be deduced that each plant has a tolerance for different Pb levels, which affects the SOD activity. Similarly, Pb levels in the branches of *C. zippeliana* from Segara Anakan were relatively high (Figure 3), which corresponded to high SOD activity. Meanwhile, even though the Pb concentration in roots was higher than that in leaves, SOD activity in roots was lower than that in leaves. The ability of Pb to induce ROS accumulation might cause a change in the activity of the SOD enzyme.

It is widely accepted that Cd toxicity causes oxidative stress in plants by causing the formation of ROS (Tiryakioglu et al. 2006). Cd can cause oxidative stress in plants by increasing ROS production (Luo et al. 2011). Plants can be protected from oxidative damage by increased SOD activity after being exposed to low Cd doses. SOD activity, on the other hand, decreases when exposed to high doses of Cd (Tiryakioglu et al. 2006). As a comparison, in leaves and roots of *B. gymnorrhiza* and *K. candel* with treatment 1HM (1.0 mg/l Pb, and 0.2 mg/l Cd and Hg), 5HM (five times higher), 10HM (ten times higher), and 15HM (fifteen times higher). Leaf SOD activity decreased at 1HM for both species compared to the control, but increased with heavy metal concentration. SOD activity in *B. gymnorrhiza* peaked at 5HM and decreased to control levels at 15HM, but remained higher than control at 10HM (Zhang et al. 2007). SOD activity in *K. candel* peaked at 10HM and remained significantly higher than controls at 15HM. Different results were obtained in sorghum with SOD activity decreasing at moderate Cd concentrations (25 μM) and increasing at higher Cd concentrations (50-100 μM) (Hassan et al. 2020). In the other hand, *C. decandra* still has lower SOD activity than *Thespesia populneoides* and *S. apetala*, with an average of 1.49 (Vadlapudi and Naidu 2009). As a result, the level of Pb and Cd concentrations that can inhibit or increase SOD enzyme activity in plants, particularly *C. zippeliana*, is unspecified.

Aside from antioxidant enzymes, plants have other defense mechanisms. For example, immobilization or detention of Cd in the cell wall to prevent Cd from entering the cell, compartmentalization or accumulation of Cd in vacuoles to limit circulation and prevent Cd from entering the cytosol, exclusion or release of Cd through cell membrane diffusion, and formation of Cd into complex compounds in the form of phytochelatins, as well as to prevent the free circulation of Cd in the organ. Metal-binding to sulfhydryl or thiol groups (-SH) is an example of phytochelatin because Pb and Cd have a high affinity for sulfhydryl groups. However, due to the inhibition of functional sulfhydryl groups in the action of SOD enzymes, this binding can alter antioxidant activity (Patra et al. 2011). It is possible that the sulfhydryl groups binding to Pb or Cd caused the decrease in SOD enzyme activity in *C. zippeliana* in this study.

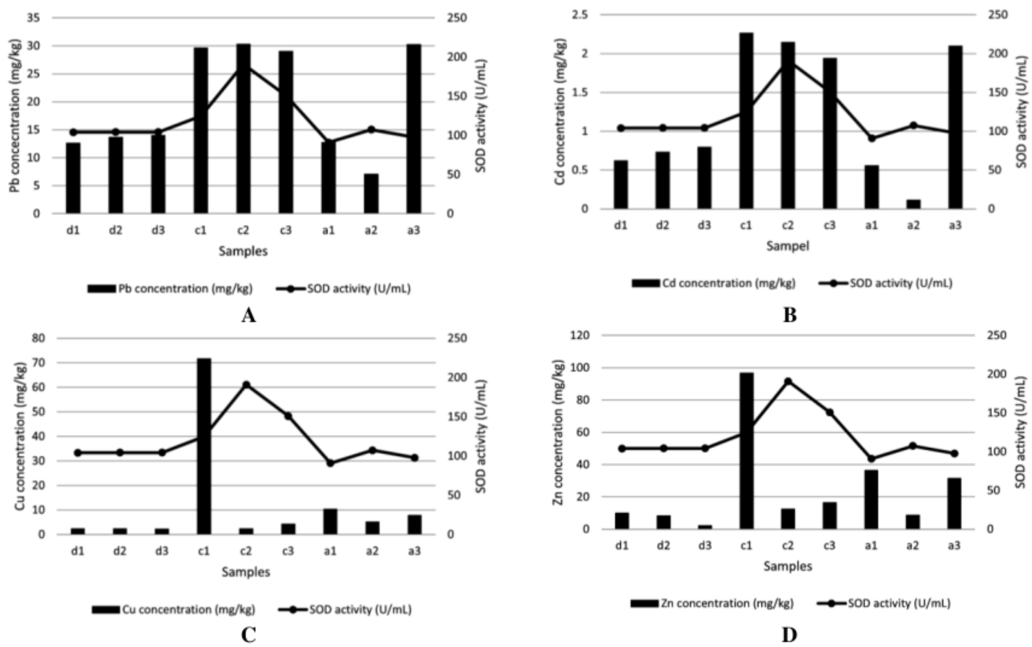

Figure 3. Correlation between heavy metal concentration and SOD activity in *Ceriops zippeliana*. A. Pb, B. Cd, C. Cu, D. Zn

Cu content is also reported as one of the factors influencing the increase in SOD activity. Cu content in leaves and roots increased with increasing Cu concentration due to treatment. It also affects the decrease in Fe and Mn content in leaves and roots (Karimi et al. 2012). The increase in Cu concentration was also accompanied by an increase in SOD activity active against ROS (Karimi et al. 2012). In this case, Cu acts as a cofactor for the SOD enzyme. Although Cu is an essential metal needed by plants, Cu can also be harmful to plants in high concentrations. Cu content that inhibits SOD activity in *Jatropha curcas* L. was 800 µmol (Gao et al. 2008), therefore it may be stated that Cu in *C. zippeliana* from Segara Anakan has not reached the level that inhibits SOD activity.

With an increase in Zn concentration in plants, the activity of SOD can increase. This can be explained by the fact that the presence of Zn as a cofactor is required for SOD (Castillo-González et al. 2018). SOD can work adequately when Zn is available in appropriate amounts. In order to increase enzyme activity, the duration of Zn treatment is also essential (Bharti et al. 2014). Similarly, a Zn deficit might reduce SOD activity. In this case, the Zn deficit mainly influences the decline in Cu/ZnSOD activity, but Zn deficiency has little effect on MnSOD activity. Even if the Zn concentration is the same, the symptoms of deficiency can be of varying severity. This is due to a differential in the quantity of Zn that is physiologically active instead of the overall Zn concentration (Cakmak et al. 1997).

Similarly, it was found in this study that the increase in SOD activity of *C. zippeliana* could be due to the availability of sufficient amounts of Zn. However, it could also decrease due to a lack of physiologically active Zn. Furthermore, according to Balafrej et al. (2020), because Zn and Cd have competitive interactions, the presence of Zn in organisms can prevent Cd toxicity. On the other hand, if an organism is lacking in Zn, Cd toxicity might rise, which is, of course, linked to an increase or decrease in SOD activity. Other factors that are thought to influence SOD activity include salinity (Carrasco-Ríos and Pinto 2014), pH (Kushkevych et al. 2014), or the presence of inhibitors such as H2O2 compounds (Hasanuzzaman et al. 2020), KCN, methyl viologen (Takagi et al. 2016), or nitration reactions that may occur in the plant body (Holzmeister et al. 2015).

It could be concluded from this study that the levels of Pb, Cu, and Zn metals in the sediments of Segara Anakan Cilacap were still within acceptable limits, while that of Cd was considered at polluting level though not excessive. *C. zippeliana* in Segara Anakan showed branches as the most metal accumulating parts compared to roots and leaves. The SOD activity in *C. zippeliana* from Segara Anakan showed a complex interaction with some factors other than metal concentrations in plant sections.

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