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Short communication: Anatomical changes in the roots, rhizomes and leaves of seagrass (*Cymodocea serrulata*) in response to lead

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Abstract. *Rosalina D, Herawati EY, Musa M, Sofarini D, Risjani Y. 2019. Short communication: Anatomical changes in the roots, rhizomes, and leaves of seagrass (*Cymodocea serrulata*) in response to lead. Biodiversitas 20: 2583-2588.* Runoff of heavy metals as a result of urban and industrial development is a potential threat for seagrass populations in the coast. The objectives of this study were to study the anatomical changes in the tissues of roots, rhizomes, and leaves of seagrass *Cymodocea serrulata* in response to treatment with different concentrations of lead (Pb) for different time durations. This experiment used heavy metal Pb from a solution of Pb $(NO_3)^{2-}$ with a concentration of 0 ppm, 5 ppm, 10 ppm, and 15 ppm and the treatment period extended up to 4 weeks with 3 replications. Analysis of changes in anatomical features showed that exodermis and endodermis cells in the roots thickened as lead concentration increased. The air spaces in the root cortex and rhizome also widened. Thickening of cell walls occurred in the epidermis and endodermis of rhizome. Likewise, in the leaves, thickening occurred in the upper and lower cuticle and also the upper and lower epidermis. In general, changes in anatomical features of root, rhizome, and leaves were observed in response to increasing lead concentrations. The results showed that *C. serrulata* developed some level of tolerance to heavy metals, especially lead.

Keywords: Accumulation, anatomy, *Cymodocea serrulata*, lead

INTRODUCTION

The phytotoxic effects of heavy metals in plants were seen in visual symptoms such as chlorosis, necrosis and wilt through a reduction in growth and accumulation of biomass (Marques et al. 2000; Sanitá di Troppi and Gabbrielli 1999). Physiological effects also noted in plants exposed to contamination, at various levels of photosynthesis, including chlorophyll biosynthesis (Chugh and Sawhney 1999). When heavy metals penetrated the roots, they partially accumulated and were translocated in cell wall (MacFarlane and Burchett 2000; Rosalina et al. 2018), with exodermis and endodermis in seagrass plant tissues which were effective barriers to the movement of these ions (Ederli et al. 2004; Lux et al. 2004; Wójcik et al. 2005; Rosalina et al. 2018).

Accumulation and localization of Cd appeared as dense granules in the roots of *Agrostis gigantea* and *Zea mays* (Rauser and Ackerley 1987), as cell wall deposit on the roots of *Zea mays* (Khan et al. 1984) and *Phaseolus vulgaris* L. cv. Contender (Vazquez et al. 1992b). Metal absorption and accumulation at higher concentrations could be cytotoxic in some plant species, causing structural and ultrastructural changes that affected plant growth and physiological well-being (Barceló et al. 1988; Vazquez et al. 1992a; Zhao et al. 2000; Han et al. 2004). Zn hyperaccumulation resulted in mesophyll cell size decrease in *Arabidopsis haleri* (Zhao et al. 2000) while Cd accumulation caused the breakdown of chloroplasts in *Phaseolus vulgaris* L. ev. Contender (Barceló et al. 1988) and reduced plant growth in *Brassica juncea* (Haag-Kerwer et al. 1999).

Brix and Lyngby (1983), and Nienhuis (1986) stated that seagrasses had the ability to accumulate heavy metals in marine waters. Seagrasses absorb heavy metals present in sea waters through their leaves, rhizomes, and roots, and they could also be used as bioindicators to monitor the presence of heavy metals (Pulich 1980; Lyngby and Brix 1982, 1983; Nienhuis 1986; Ward 1989). Further study of the anatomical changes that occurred in seagrass tissue (roots, rhizomes, and leaves) in response to heavy metals would help to understand the process of their accumulation and also heavy metal tolerance.

MATERIALS AND METHODS

This research was carried out in February 2018. Healthy seagrasses belonging to the species *Cymodocea serrulata* having the same number of leaves and vertical internodes which look almost similar were used in this study. These sample seagrass plants had 3-4 leaves whose width was 0.5-0.8 cm and length was 6.2-22.5 cm. The experimental

specimens were placed in 12 aquariums were 30 cm long, 30 cm wide and 25 cm high. Each of them was filled with 30 seagrasses for 4 weeks. The plants were exposed to lead from $Pb(NO_3)_2$ solutions at 0, 5, 10, and 15 ppm concentrations for 4 weeks, with 3 repetitions for each treatment. They were observed at the 1st, 2nd, 3rd, and 4th week, in accordance with Tupan and Azrianingsih (2016). The research was conducted at the Biology Laboratory, Faculty of Mathematics and Sciences, University of Brawijaya, Malang, Indonesia. anatomical observations and photomicrography of seagrass anatomical sections were carried out at the Biomolecular Laboratory, Faculty of Mathematics and Sciences, University of Brawijaya, Malang, Indonesia.

Procedures: Histological preparations

To test seagrass tissue histologically, *C. serrulata* seagrass samples were taken out from experimental aquariums every week. The seagrass parts were separated as roots, rhizomes and leaves, cleaned with distilled water and then immersed in a solution of FAA (90 ml from 70% ethyl alcohol: 5ml formaldehyde: 5ml acetic acid) which functions as a fixative. The preparation was according to modified method of Ruzin (1999). The histological preparations used fresh preparations fixed and cross-linked using stainless steel and placed between two cassava or carrot cork blocks using a microtome (Euremex MT.5503 clamp on the hand microtome). After being cut thin, the sections were immersed 1% safranine for approximately 5 minutes. After safranine staining, the sections were rinsed with distilled water to remove any impurities attached to the sections. The sections were then mounted on slide in glycerin, and covered a glass cover. The anatomical observations were performed using a light microscope. Measurement of thickness of exodermis and endodermis of root, thickness of epidermis and endodermis of rhizome, as well as thickness of cuticle and epidermis of both abaxial and adaxial section of leaves was performed using the Olympus BX51 Digital Imaging Microscope.

Data analysis

Result about the thickness of the exodermis and endodermis of root, thickness of the epidermis and endodermis of rhizome, as well as thickness of the cuticle and epidermis of both abaxial and adaxial side of leaves were analyzed using One Way ANOVA at 95% significance ($p < 0.05$). If the results showed a significant difference, then proceeded with the Least Significant Difference Test (LSD) at the level of 95%. Data were analyzed using SPSS version 21 program. Data were tested for normality, homogeneity, and non-additives before analysis, as a condition for ANOVA test.

RESULTS AND DISCUSSION

Anatomical changes of *Cymodocea serrulata* roots in response to lead

The effect of different concentration of lead on the thickness of exodermis and endodermis of roots of *C. serrulata* is given in Table 1. The effect of 15 ppm of lead, when compared to control (0 ppm) on the anatomical features of roots, is shown in Figure 1.

Accumulation of lead in the root tissue of *C. serrulata* showed changes in the tissues affected by lead. Exodermis and endodermis thickness changed with increasing lead concentration and length or duration of exposure. Increased concentration lead resulted in a decrease in the thickness of exodermis of the roots. According to Al-Saadi et al. (2013), lead metal accumulation increased along with increasing concentration of heavy metals. During stress, the root growth rate decreased, and exodermis and endodermis would develop closer to the root tip, indicating that stress accelerated the development of exodermis and endodermis (Enstone et al. 2003). On the other hand, reduction in root growth happened due to a decrease in cell division as a result of increased cell wall thickness exposed to heavy metals (Stohs et al. 2000). The higher of the lead cause the thicker exodermis and endodermis cells in the seagrass roots (Figure 1). This showed that lead metal accumulation could accelerate the maturation of exodermal and endodermal cell walls. According to Enstone et al. (2003), the root with mature exodermis is a barrier to the entry of apoplast ions found near the root surface. Changes in size, and cell form showed disturbances in heavy metals in cell maturation at the root and disrupts hormonal balance (Barceló and Poschenrieder 1990; Sandalio et al. 2001). Metals absorbed in the cell wall and the intercellular space of the cortical parenchyma at the root was one of the strategies of seagrass for self-defense (Gomes et al. 2011).

Table 1. Thickness of endodermis and exodermis of roots of *Cymodocea serrulata* treated with different concentrations of lead

Roots tissue	Lead concentration (ppm)	Week 1	2	3	4
Exodermis thickness (μm)	0	25.41±3.09	27.17±6.09	27.95±5.53	29.29±5.10
	5	22.89±4.23	25.16±3.54	27.59±4.50	29.47±4.32
	10	26.71±4.01	27.88±4.32	28.66±3.16	30.22±3.59
	15	23.30±4.45	26.01±2.07	26.37±4.64	28.04±4.13
Endodermis thickness (μm)	0	6.52±1.27	8.40±1.07	9.10±1.22	9.25±1.35
	5	7.48±1.50	8.22±1.19	9.26±1.52	9.30±1.22
	10	8.94±1.08	9.81±1.37	10.09±1.65	10.24±1.54
	15	9.44±1.26	9.94±1.74	9.92±1.72	10.64±1.60

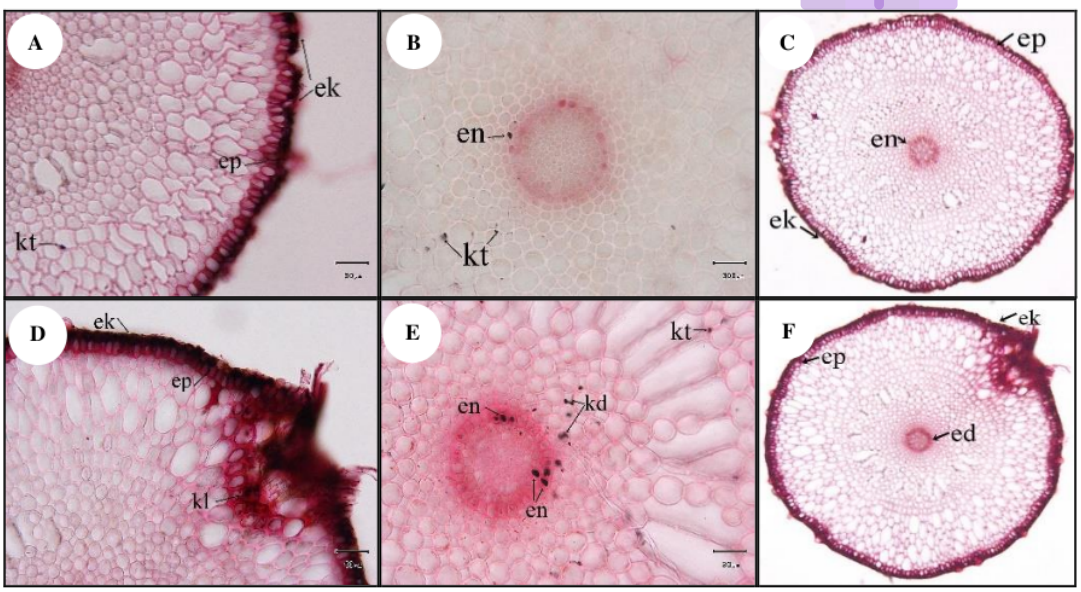

Figure 1. Anatomical features of roots at 15 ppm of lead treatment and control (0 ppm); A, B, C show thickness of ek (exodermis), ed (endodermis) and kt (middle cortex) in the control (arrow). D, E, F show changes in thickening of ek (exodermis), ed (endodermis), kt (middle cortex), kd (inner cortex) at 15 ppm lead treatment (arrow)

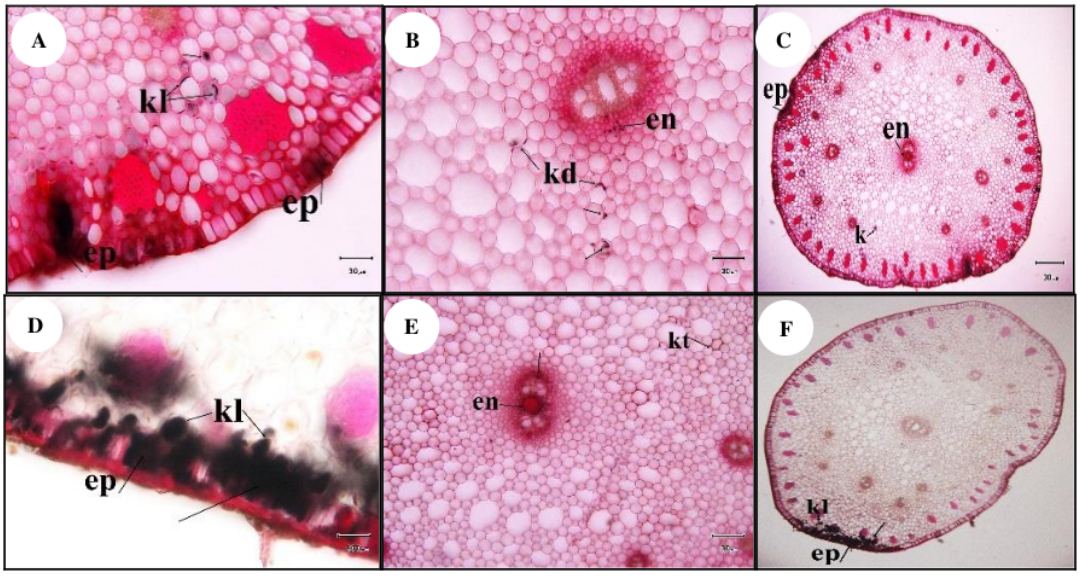

Figure 2. Changes in anatomical structure of rhizome at 15 ppm lead treatment, compared with control (0 ppm); A, B, C shows changes in ep (epidermis), ed (endodermis), kd (inner cortex) and airspace (cortex) in the control (arrow). D, E, F shows changes in thickening of ep (epidermis), ed (endodermis), kl (outer cortex) at 15 ppm lead treatment (arrow)

Anatomical changes in *Cymodocea serrulata* rhizome in response to lead

The concentration of 5 ppm of lead resulted in the lowest change of exodermis structure in the epidermis which was significantly different from the changes at a concentration of 10 and 15 ppm. However, it was not significantly different from the concentration of 0 ppm. It was found that the changes in the 2nd week of treatment were lower than that in the 4th week (Table 2). The effect of lead on the anatomy of seagrass rhizome is shown in Figure 2.

Increased concentration lead resulted in increase in thickness of the epidermis and endodermis of the rhizome. Changes in epidermis and endodermis tissue and thickening of the cell wall layers (Figure 2) were the result of accumulation of heavy metals in seagrass epidermal and endodermis tissues resulting in faster cell maturation and thickening of tissues. Thickening of epidermis and endodermis cells in the rhizome was an adaptation to prevent the translocation of lead metal to other tissues. Cortex cells in the rhizome showed changes in the air space. Air space was enlarged due to reduce diaphragm.

Cortex cells consisted of pseudohypodermis and aerenchyma tissue. According to Al-Saadi (2013), changes in size, shape of cortical parenchyma cells occur due to the accumulation of heavy metals. The higher concentration of heavy metals would expand the cell space in the parenchyma cells of the cortex and reduce the vascular bundles. In *Potamogeton crispus* and *P. perfoliatus*, the thickening of the walls of both xylem elements and tissues accumulating metals, and of cortical parenchyma thickening were the other anatomical adaptations for heavy metal toxicity (Vazquez et al. 1992).

Anatomical analysis of *Cymodocea serrulata* leaves

In the abaxial cuticle of seagrass leaves, the lowest average change in thickness was observed at the concentration of 5 ppm and it was significantly different from that of 15 ppm which was the highest (Table 3). Duration-wise, the average change in the abaxial cuticle thickness was the lowest in the 1st week and it was significantly different from that of the 4th week, which was the highest (Table 3).

Table 2. Anatomical changes in rhizome of *Cymodocea serrulata* exposed to lead

Rhizomes tissue	Lead concentration (ppm)	Weeks			
		1	2	3	4
Epidermis thickness (μm)	0	30.55±4.45	38.30±4.38	40.22±6.24	41.73±7.08
	5	33.92±4.75	36.26±5.44	37.47±10.65	38.43±5.33
	10	37.14±6.34	38.51±3.94	39.61±7.26	44.64±8.07
	15	40.10±4.61	40.44±5.54	40.89±9.47	49.26±8.64
Endodermis thickness (μm)	0	12.02±2.23	15.44±2.02	15.90±2.45	17.09±1.94
	5	13.61±2.71	13.64±1.87	14.95±1.72	15.77±2.85
	10	17.39±1.87	14.36±2.26	15.92±2.21	17.39±1.87
	15	17.72±1.82	14.82±1.95	17.92±1.78	17.73±2.42

Table 3. Anatomical changes in leaves of *Cymodocea serrulata* exposed to lead

Leaf tissue	Lead concentration (ppm)	Duration of exposure in weeks			
		1	2	3	4
Cuticle abaxial (μm)	0	4.82±0.884	5.27±0.69	5.30±1.61	5.43±1.01
	5	4.07±0.697	4.09±0.68	4.50±0.87	4.93±1.51
	10	4.38±0.796	4.56±0.66	5.17±0.87	5.62±0.77
	15	4.22±0.723	6.09±1.16	6.16±1.12	7.04±1.82
Cuticle adaxial (μm)	0	3.97±1.17	5.358±0.84	5.43±1.02	5.52±1.02
	5	3.87±0.76	4.14±0.73	4.20±0.82	4.60±0.99
	10	4.12±0.68	4.37±0.74	4.83±1.21	5.89±1.07
	15	4.42±0.68	5.49±1.48	6.08±0.68	6.28±1.78
Epidermis abaxial (μm)	0	11.02±2.77	11.10±1.64	11.42±2.14	11.82±1.33
	5	11.30±2.38	11.79±2.47	11.82±2.13	12.61±2.15
	10	10.99±2.29	11.47±2.57	12.01±4.64	13.02±1.94
	15	11.27±8.33	11.38±.2.87	12.97±1.72	13.45±1.99
Epidermis adaxial (μm)	0	10.35±1.47	11.55±2.07	12.412±2.011	12.58±2.607
	5	10.913±2.495	11.248±1.516	11.613±1.719	12.839±1.932
	10	8.979±1.510	11.558±2.367	11.960±1.642	13.317±1.995
	15	10.975±1.334	12.471±1.675	12.828±1.474	13.018±2.115

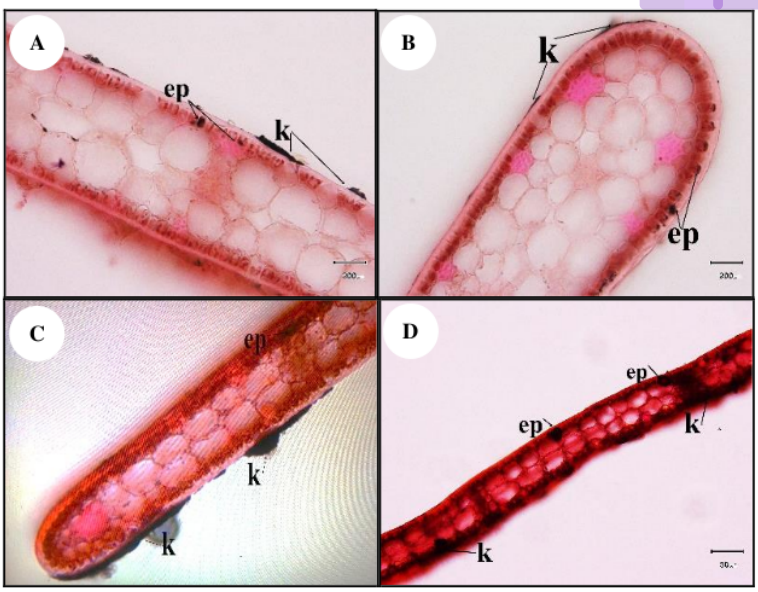

Figure 3. Changes in leaf anatomy at 15 ppm lead, compared with control (0 ppm); A. Cuticle in abaxial and adaxial (k), epidermis in abaxial and adaxial (ep) surfaces in the leaf of control plants(arrow). B. Nature of cuticle in abaxial and adaxial (k), and epidermis in abaxial and adaxial (ep) at 15 ppm lead treatment (arrow)

Adaxial cuticles were the lowest at 10 ppm compared to the highest at 15 ppm concentration. Duration-wise, average changes in the adaxial cuticle thickness in the Ist week was lowest in comparison to the 4th week which was the highest (Table 3). Concentration of 0 ppm produced the lowest average change in the thickness of abaxial epidermis compared to the highest change observed in concentration of 15 ppm. It was lowest in week 1 period compared to the highest in week 4 concentration of 10 ppm resulted in the lowest changes in the adaxial epidermal structure compared to 15 ppm (Table 3). The effect of lead on leaf anatomy can be seen in Figure 3.

Analysis of anatomical structure in seagrass leaves in response to lead showed changes in the cuticle layer and thickening of epidermis cells according to the concentrations of lead (Figure 3). Increased concentration lead resulted in an increase in the thickness of the abaxial cuticle and increased concentration of lead resulted in an increase in adaxial epidermal thickness. Aquatic plants submerged in water could absorb nutrients, including metals, through their parts including leaves and absorption was influenced by the structure and permeability of cuticle layer. The mechanism of the entry of lead metal into leaf tissue is through leaf stomata because the size of leaf stomata was greater than the size of lead metal (Tomlinson 1980; Kuo 1983). The seagrass species *C. serrulata* did not have any stomata but had thin and oval, hollow cuticle. This cuticle served as the entry pathway for lead metal into seagrass leaves. The thickenings developed in the cuticle and epidermis of seagrass in response to lead may be one of the self-defense mechanisms. According to Sandalio (2001), heavy metals could induce oxidative stress. In the leaves of *Pisum sativum,* physiological responses to heavy metals related to oxidative stress, such as cell disorders characterized by increased mesophyll cell size, reduction in inter-cell space and disruption in the structure of chloroplasts (Sandalio 2001). Shaw (1995) reported that heavy metals Cd and Hg resulted in detrimental effects, especially on the membrane, in *Phaseolus aureus*. Al-Saadi (2013) showed that *Potamogeton* leaves responded to the presence of heavy metals by a reduction in epidermal cell size and aerenchyma tissue.

In conclusion, lead was found accumulating in every part of seagrasses (*Cymodocea serrulata*) such as roots, rhizomes and leaves and the higher the concentration of lead, thicker was the tissue, especially the epidermis and endodermis. This may be one of the strategies of seagrass to minimize the spread of heavy metal to other tissues.

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Wrong Article You may have used the wrong article or pronoun. Proofread the sentence to make sure that the article or pronoun agrees with the word it describes.

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S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agrees with the verb.

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Article Error You may need to use an article before this word.

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S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agrees with the verb.

Article Error You may need to remove this article.

Article Error You may need to use an article before this word.

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Article Error You may need to use an article before this word. Consider using the article **the**.

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PAGE 3

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Article Error You may need to use an article before this word. Consider using the article **the**.

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Article Error You may need to use an article before this word.

Article Error You may need to remove this article.

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Article Error You may need to use an article before this word.

Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.

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Missing "," Review the rules for using punctuation marks.

Article Error You may need to use an article before this word.

Article Error You may need to use an article before this word. Consider using the article **the**.

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Article Error You may need to use an article before this word. Consider using the article **the**.

Possessive Review the rules for possessive nouns.

Missing "," Review the rules for using punctuation marks.

Article Error You may need to use an article before this word.

P/V You have used the passive voice in this sentence. You may want to revise it using the active voice.

Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.

Wrong Form You may have used the wrong form of this word.