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Anatomical Responses of Marigold (*Tagetes erecta* L.) Roots and Stems to Batik Wastewater

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Abstract. The batik industry in Yogyakarta is mostly found in small or medium scale industries. This condition results in a limited capacity for waste processing. Batik wastewater contains various types of heavy metals such as chromium, aluminium (Al) and iron which can be dangerous to the environment. Marigold (*Tagetes erecta* L.) as a member of the Asteraceae family is known to have potential as a phytoremediator plant of various types of metals. This study aimed to determine the anatomical responses of *T.erecta* L. roots and stems to the batik wastewater exposure. The concentration of waste used were 0%, 25%, 50%, 75% and 100%. The treatment was done every five days for 25 days according to field capacity to one and a half months old plants. Parameters observed were plant morphology, plant growth, root and stem anatomy. The preparation of transverse section of roots was carried out by paraffin embedding method, while the transverse section of stems was prepared by non-embedding method. The results showed that there was a decrease in plant height, roots volume, root length, and plant biomass along with increasing concentration of the wastewater given. The leaves growth increased most rapidly at 25% waste. The treatment of 50% to 100% waste caused leaf chlorosis and necrosis and damage to the root epidermis and cortex cells as seen by cells shape, from round or pentagonal or hexagonal to ellipsoid cells. The highest values of root, stele, and trachea diameters, as well as cortex thickness, were obtained in the concentration of 50% while the lowest was found at 100%. The stem diameter increased up to 50% treatment with the highest value and then decreased by 75% and 100% waste. The stem epidermis thickness, cortex thickness, and vascular tissue were reduced in treated plants, with the lowest was found in 100%.

INRODUCTION

Yogyakarta city is well known as the center of batik in Indonesia. The batik industry is more often found in small or medium scale industries. This condition has many benefits for local people but also results in a limited capacity for processing waste. Most batik industries in Yogyakarta city were still disposing of the wastewater carelessly in the environment without going through a waste treatment process by licensed regulation related to this matter [24].

Batik industry is one of textile industries that yield waste from its colouring process. The amount of liquid waste from batik processing could reach 80% of the total water used [12]. Based on research by Sasongko and Tresna [32], it is known that batik waste contains heavy metals such as chromium (Cr), copper (Cu), aluminium (Al), and iron (Fe) [30]. Heavy metal is non-degradable and persistent in the soil and could enter the food chain if not carefully treated. Exposure to heavy metal contamination, no matter how small, could be harmful to the organism [29].

Living organisms need heavy metal as a part of their nutrition. For example, human needs iron, cobalt, copper, manganese, molybdenum. and zinc. Nonetheless, all those heavy metals could turn toxic if consumed at high concentrations [35]. Those heavy metals are being absorbed by plants and causing oxidative stress leading to cellular damage and homeostatic and ionic disturbance. When the plants are being eaten by the livestock it could damage their internal organs because the heavy metal elements are being disposed of at the liver, kidney, muscle and other tissues [34].

Based on the detrimental impact of heavy metal waste, it is undoubtedly necessary to solve this environmental problem. The resolving solution could be based on cleansing the waste, whether it is physically, chemically, or even biologically using certain plants or microorganisms. The use of plants to remove heavy metal contamination and

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restore soil quality is called phytoremediation. This method is preferable due to its considerably lower cost of operation [22].

Plants of the family Asteraceae are known for having potential as phytoremediation agent for their hyperaccumulator trait, that is the ability to accumulate metal inside its root and stem and can be used for phytoextraction function [19]. The types of metal that can be well accumulated by plants from family Asteraceae are strontium (Sr), cesium (Ce), and uranium (U) [9]. *Dicoma niccolifera* is known as a species for its ability to accumulate chromium in high concentrations inside its tissue [28]. This research project aimed to observe the anatomical response from Marigold (*Tagetes erecta* L.) toward liquid waste exposure from the colouring process.

METHOD

Analysis of batik liquid waste content

The concentration of heavy metal contamination for Fe, Cr, Cu, and Cd were analyzed at BBLTKLPP (Center for Environmental Health and Disease Management, Yogyakarta), meanwhile for contamination of Al was analyzed at The Laboratory of Analytical Chemistry at Faculty of Natural Sciences and Mathematics, UGM.

Field capacity measurement

Field capacity measurement was carried out to determine the volume of water that optimally absorbed on the soil. That volume was then used to determine the volume of watering the plants. First, the planting media was weighed about 1 kg, then was dried in the oven at a temperature of 55° C until it reached constant weight. After constant weight was gained, 300 g of the planting media was put into a 10 x 20 cm polybag. A 500 mL beaker was filled with water, then a polybag containing planting media was placed on top of a 1000 mL empty beaker. Water from the 500 mL beaker was poured onto the polybag until it ran ut and stops dripping from the polybag to the beaker below. The difference in the volume of water accumulated in the 1000 mL beaker and the volume of water poured in the field capacity of the planting media.

Preparation of batik liquid waste

The result of field capacity previously obtained (400 ml) was used as a reference volume that was poured onto the treated plants. The treatments are a mixture of water and liquid waste divided into 5 categories, namely 0%, 25%, 50%, 75%, and 100%.

Planting *Tagetes erecta L.* and treatment

T. erecta L. seeds were soaked in warm water for ± 30 minutes, drained, then sown in soil media in a 17 × 35 cm polybag by placing them on top of the media with 2 cm distance between seeds. The polybags containing the seeds were placed in a shady place and were not exposed to direct sunlight. The seeds were watered according to the need to maintain moisture. After two weeks, the seedlings were transferred to a 10 × 20 cm polybag, one seedling per polybag then acclimatized for one week before the treatment given.

Wastewater Treatment

The mixture of water and liquid waste with a concentration of 0%, 25%, 50%, 75%, and 100% was poured to the planting media. Watering was done every five days for 25 days of observation. Marigold plants were harvested after 25 days of treatment by uprooting the entire plant

Preparation of transverse sections of *Tagetes erecta* L. root

The preparation of root transverse sections was carried out by cloaking the cutting of the root in paraffin (embedding). The main root was cut 1 cm from the tip, then fixed using FAA solution which is a mixture of formalin, glacial acetic acid, and alcohol in the ratio of 5:5:10 for 24 hours. The root cuts were stained by soaking them in safranin 1% in 70% alcohol for 24 hours, continued by removing the fixative solution and substituted it subsequently with alcohol of 70%, 80%, 95%, 100% I, and 100% II respectively for 30 minutes each. Dealcoholization was finished by soaking the samples into a mixture of alcohol: xylol in a ratio of 3:1, 1:1, and 1:3 and last into xylol only for 30 minutes, twice. The specimen was then put into a xylol: paraffin mixture in the ratio of 1:9 at 57° C for 24 hours. The xylol-paraffin solution was removed and then replaced with pure paraffin at the same temperature and time range. Paraffin was discarded and replaced with new pure paraffin. After 1 hour, paraffin containing specimens were cut into blocks. The block containing the sample is sliced using a rotary microtome with the thickness of 6-12 µm. The slice was glued to the microscope slide using a mixture of glycerin: albumin spiked with water, then placed on a hot plate at temperature of 45° C until the paraffin band is stretched. After drying, the specimen slices were smeared with Canadian balsam and covered with a glass cover. The finished specimen was observed under a microscope that has been connected to a computer equipped with Optilab and Image Raster software.

Preparation of transverse sections of *Tagetes erecta* L. stem

The transversal sections of stem were done by cutting the stem using a sliding microtome. The sample then kept inside 100% alcohol, and stained in safranin 1% in 70% alcohol for 24 hours, continued by immersing them in an alcohol of 70%, 80%, 95%, 100% I, and 100% II solution respectively for 3 minutes each, then finished by soaking the sample into the mixture of alcohol:xylol in ratio of 3:1, 1:1, and 1:3 and last into xylol only for 30 minutes, twice. The cutting stem was then put in the microscope slide and smeared with Canadian balsam then covered with a glass cover. The finished specimens were observed under a microscope that has been connected to a computer equipped with Optilab and Image Raster software.

Research Parameters

Anatomical quantitative parameters of roots and stems observed were epidermal thickness, cortex thickness, stele diameter, trachea diameter, and diameter of roots and stems. The qualitative parameters observed were a comparison of the anatomical conditions of roots and stems in the treatment and control described descriptively.

Morphological and growth parameters measured were root length, number of roots, wet weight and root dry weight, and plant height. Descriptively, the comparison of root and stem conditions in the treated and control plants was observed by taking photos of the roots and stems as a whole and the details of the root strands using a stereo microscope.

RESULTS AND DISCUSSION

From the standardized test in BBTKLPP and Laboratory of Analytical Chemistry, it was revealed that liquid waste from Central of Batik Halus "Arif" within the area of Tegal Rejo, Girirejo, Imogiri, Bantul contained several heavy metal contaminants (Table 1).

TABLE 1. The Analysis of Metals Content in Batik Liquid Waste

Parameters	Method	Results (ppm)	Quality standard (ppm)
Total Chrom (Cr)	SNI 6989.17-2009	<0.0213	2
Copper (Cu)	SNI 6968.6-2009	0.0192	0.3
Iron (Fe)	SNI 6989.4-2009	0.2230	5
Cadmium (Cd)	SNI 06-6989.38-2005	<0.0034	0.05
Aluminum (Al)	*Atomic Absorption Spect.*	11.869	0.2*

* The metal amount has exceeded the quality standard

The type of metal found with the greatest concentration was Al, and this value was followed by Fe and Cr. Al metal content has exceeded the water quality standard set by the Ministry of Health in Permenkes No. 492/2010, while the level of Cr, Cu, Fe and Cd has not exceeded the limit in the quality standard of batik industrial waste determined by the D.I.Yogyakarta Regional Regulation No. 7/2016. [27].

Plant Morphology

Based on the appearance of habitus, it can be seen that in general, marigold plants tend to have shorter posture, more pale leaves colour, and smaller stem as the concentration of waste increased (Figure 1). Flower buds were only found in control and 50% treatment. This condition is likely to occur due to increasingly damaged roots as the concentration of waste increases (Figure 1C). Probably, Al caused disturbances in the root system (Figure 1C) that make it difficult for plants to absorb nutrients leading the growth inhibition. Ciamporová [4] revealed that the most important effect of Al toxicity is related to roots, where Al inhibits cell division or interferes with nucleic acid activity at the root tip. Al becomes dangerous in easily dissolved conditions such as in the ion form (Al^{3+}), the change into to Al^{3+} form is caused by low pH or acidic soil [25], while the pH of planting media in this study was ≥8. Brautigan *et al*. [3] states that Al toxicity can still occur in alkaline soils and can inhibit root growth and reduce the productivity of *Pisum sativum* L. var Santi. In this case, the toxicity was not caused by Al^{3+} but by the anion form $Al(OH)^{4-}$.

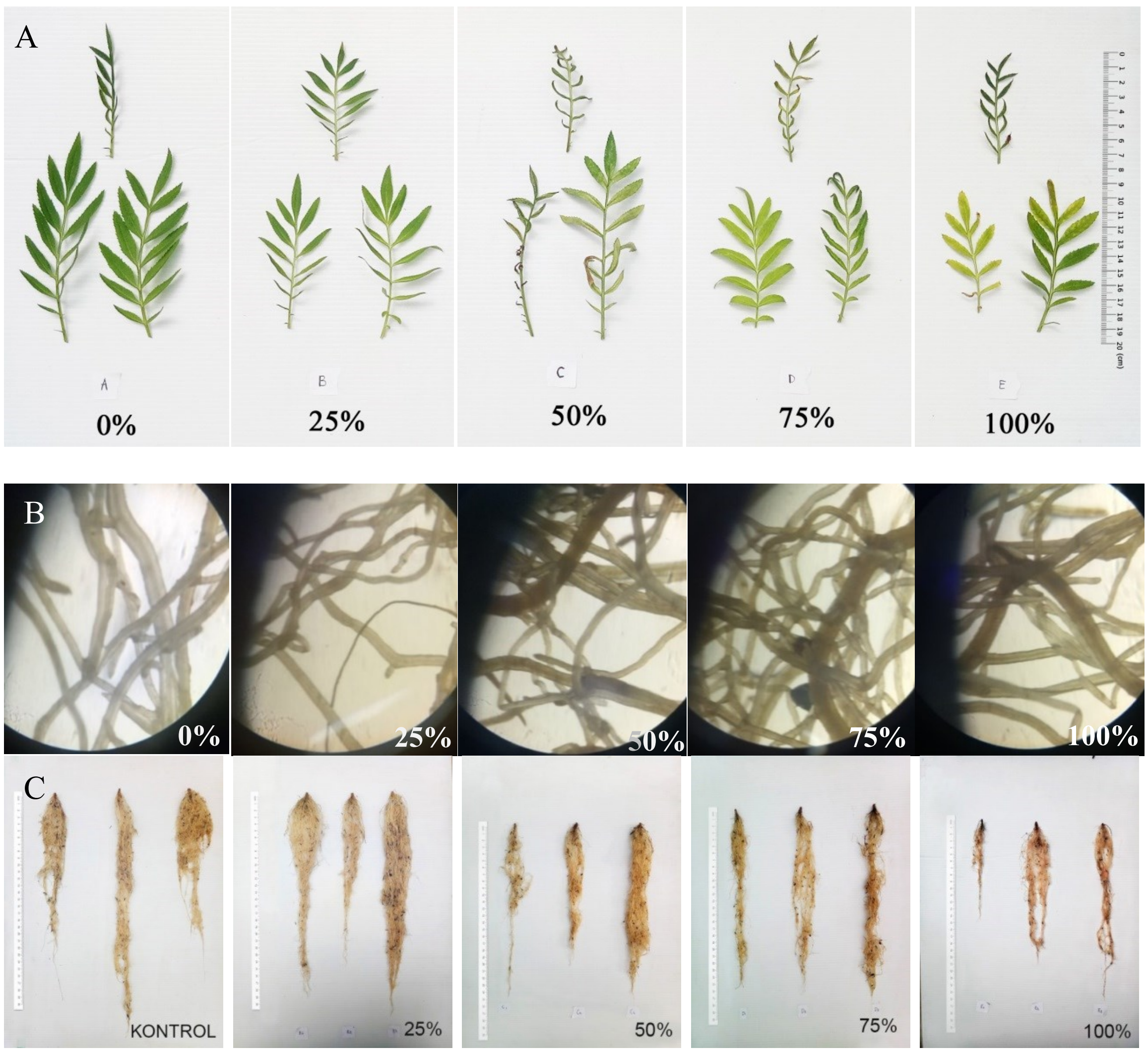

FIGURE 1. The plant morphology of Marigold plant after 25 days of batik wastewater treatment with different concentration (A: leaf morphology; B: photos of root with a stereo microscope; C: root morphology)

Figure 1A shows the detailed condition of the Marigold plant leaves. The width of the compound leaflets was narrower, the colour of the leaves also appeared increasingly yellow as the waste concentration increased. Some copper-white patches were found on the leaves of 50% to 100% treated plants. The higher the concentration of waste, the more leaves were found with a rough texture, wavy, curled or blackish colour similar to burned leaves. According to Lee *et al.* [20] patches or creamy or white lesions are one of the symptoms of chlorosis, while curly and blackishyoung leaves were the result of cell death (necrosis) [16] which was likely due to the effect of heavy metal toxicity in liquid waste of batik dyeing.

Exposure to heavy metals can interfere with the absorption of important nutrients and their distribution throughout plant tissues, this can be caused by several causes such as competition with the same transporter, disruption in water absorption, or disturbance of key enzymes in the transportation process [23]. Gupta *et al*. [11] revealed that Al stress in plants causes inhibition of absorption of cations such as Ca^{2+}, Mg^{2+}, K^{+}, NH^{4+} which results in the deficiency of these elements. Meanwhile, chlorosis and necrosis are likely related to magnesium deficiency which is the main constituent element of chlorophyll. According to Bian *et al*. [2] the condition of bumpy and curled leaves and the total damage of growing points are several symptoms of Al stress in the shoot system.

Figure 1B shows the root morphological appearance at concentration of 0% to 100% of liquid waste. Root branching was denser at higher concentration of waste. In control, the root colour was lighter, and the surface was smoother, while at higher concentrations of waste the roots appear darker and have epidermis with a rough texture. Al is not a Fenton type metal (a metal that can react with hydrogen peroxide in the Fenton reaction and produces free radicals) but clearly shows prooxidant activity, so exposure to Al metals at high concentrations can induce root oxidative stress [8]. The appearance of coarser root texture in the treatment of higher concentrations of waste is likely caused by damage to the structure of epidermal cells due to the production of ROS (Reactive Oxygen Species) by Al [14].

The root morphological appearance is shown in Figure 1C. In general, it can be seen that both the length and the thickness of the root decreased with the increase of the concentration of waste given. Root colour seems to be darker, probably because of the accumulation of dyeing agent. The roots texture also becomes harder as the concentration of waste increased. The root of control and 25% treatment appeared fresher and bloated, while roots of 50% and 75% treated plants were dry and fragile.

Growth parameters

TABLE 2. Growth Parameters of *Tagetes erecta* L. Roots After Being Treated with Batik Wastewater at Different Concentrations

Waste concentration	Plant height (cm)	Number of healthy leaves (sheets)	Root length (cm)	Root volume (mL)
0%	57.57 ± 10.18^{a}	16.71 ± 3.9^{a}	33.73 ± 4.77^{a}	10.00 ± 0.00^{a}
25%	51.36 ± 7.65^{ab}	16.86 ± 4.63^{a}	30.00 ± 4.58ab	8.00 ± 3.46^{a}
50%	47.57 ± 8.88^{ab}	15.29 ± 5.09^{a}	26.67 ± 3.21^{ab}	4.00 ± 1.00^{b}
75%	46.36 ± 9.59^{b}	16.57 ± 2.51^{a}	27.17 ± 0.76^{ab}	3.00 ± 1.00^{b}
100%	42.21 ± 9.17^{b}	17 ± 4.55^{a}	22.33 ± 5.25^{b}	1.67 ± 0.58^{b}

Numbers followed by the same letters in the same column show no significantly difference from the Duncan test with a confidence level of 95%.

The control treatment showed the highest plant height, meanwhile the 100% treatment has the lowest one (Table 2). The result obtained indicates that the higher the concentration of batik waste given, the plant growth was increasingly inhibited. Stunted plant growth is likely caused by disruption of nutrients absorption by the root because the toxicity of Al metal has a major influence on root damage [5].

Based on research conducted by Konarska [17], Al stress was also found to inhibit the development of organs above the ground in red chili (*Capsicum annuum* L.) plants. Apart from decreasing the ability of the root system, the inhibition of canopy growth can also be caused by Al disruption against the activity of important enzymes in the processes responsible for plant growth such as photosynthesis and respiration [21].

Cr was also considered to play a role in inhibiting the growth of marigold plants in high concentrations of waste. Cr was toxic to most higher plants at concentration 100 $\mu M\ kg^{-1}$ or equal to 0.1 ppm [6]. Cr concentration contained in the waste was 0.0213 ppm. This value has not exceeded the standard threshold of liquid waste quality, but the watering treatment was done as much as 5 times and given that heavy metals cannot be degraded in the soil. It is assumed that the Cr metal absorbed by the plant is several times higher than the amount contained in the waste.

The decrease in the number of fresh leaves in the 50% treatment was due to a large number of mature leaves turned yellow and dry. This condition also occurs in 75% and 100% treatment, but it is followed by the formation of new leaves. The 100% treatment has the highest number of leaves replenishment. The provision of waste is somehow spurred leaf formation. However, the leaves formed are smaller and have chlorosis and necrosis.

Metal stress can inhibit metabolism in the leaf area due to the disruption of cell division, photosynthesis, respiration and protein synthesis [4]. Rout *et al*. [31] revealed that Al toxicity appears through the symptoms of calcium (Ca) deficiency due to disruption in the process of transporting Ca. Symptoms of Ca deficiency include curling or folding young leaves and total damage of growing points. Almost all at 50% to 100% treatment shows symptoms of Ca deficiency, both from the colour of the leaves that turn yellow (chlorosis), curly or folded leaves, and total damage of growing points indicated by leaf buds that looks like burning.

The control group has the highest value of root length and volume, while the 100% treatment had the lowest root length and volume among all treatments. The decrease in root volume is proportional to the decrease in root length, where the more hampered root growth causing the number and the volume of root even smaller.

Jones *et al*. [13] stated that Al in toxic concentrations increases cell wall stiffness by disrupting Ca^{2+} homeostasis on the cell wall. Calcium (Ca) is one of the essential elements for plants. The form of divalent cation (Ca^{2+}) is needed for the structural role in cell walls and membranes, as a counter-cation for inorganic and organic anions in vacuoles, as well as intracellular couriers in the cytosol. In addition to Al, Cu metal is also known to increase cell wall stiffness which results in a decrease in the ability of root cell elongation [18].

Plant Biomass

Plant productivity can be identified by measuring the weight of fresh and dry roots and plant shoots. Dry weight is the result of photosynthates stored in plant organs, while wet weight is still influenced by the presence of water content in plant organs. Based on the results of measurements of biomass marigold plants treated with batik wastewater for 25 days the results obtained as shown in Table 3.

TABLE 3. Plant Biomass of *Tagetes erecta* L. after Being Treated with Batik Wastewater at Different Concentrations Numbers followed by the same letters in the same column show no significant different from the Duncan test with a confidence level of 95%.

Waste treatment	Root Fresh weight (g)	Root Dry weight (g)	Shoot Fresh weight (g)	Shoot Dry weight (g)
Control	6.40 ± 0.88^{a}	0.37 ± 0.05^{ab}	12.50 ± 3.06^{a}	1.32 ± 0.52^{a}
25%	7.10 ± 5.27^{a}	0.41 ± 0.29^{a}	12.27 ± 3.12^{a}	1.20 ± 0.36^{ab}
50%	3.87 ± 1.98^{a}	0.24 ± 0.14^{ab}	9.67 ± 4.37^{ab}	0.92 ± 0.42^{ab}
75%	2.53 ± 0.60^{a}	0.16 ± 0.05^{ab}	7.77 ± 1.37^{ab}	0.73 ± 0.06^{ab}
100%	2.13 ± 1.43^{a}	0.10 ± 0.62^{b}	5.50 ± 1.40^{b}	0.57 ± 0.14^{b}

The results in Table 3 show that treatment with 25% waste gives the highest yield for roots fresh and dry weight, and these values were decreased with the following treatments of 50%, 75%, and 100%. It is estimated that plants can still tolerate waste with concentration of 25%. Al concentration at 25% may be the optimal composition for beneficial impact in marigold plants. Al is known as a beneficial element, it is not essential for plants, but when given they can help plant growth and development by stimulating resistance mechanisms against biotic and abiotic stress, helping to use other nutrients or overcome the toxic effects of other elements [15].

Increasing concentration of wastewater also resulted in a decrease of the fresh and dry weight of shoots, as the highest value was found in control. The decrease in biomass under heavy metal stress conditions occurs could be due to root damage by the toxicity of Al and Cr, as indicated by the decrease in root length and root volume as the concentration of waste increased. The shorter and fewer roots a plant has, the more limited its ability to absorb nutrients and water needed for growth. The occurrence of chlorosis, necrosis, and the smaller size of leaf strands in the higher waste treatment also have a large effect on decreasing plant biomass. Chlorosis, necrosis, and the increasingly narrow size of leaf strands limit the ability of plants to carry out photosynthesis due to the low chlorophyll content and the limited exposure area at the leaves, resulting in lower plant productivity, indicated by low biomass.

The condition of alkaline media ($pH \geq 8$) may also affect reducing plant biomass. Soil pH greatly affects the solubility of the nutrient compounds in soils, biological reactions that release nutrients, and the development of toxicities from particular elements [35]. Under alkaline conditions with $pH \geq 8$, the availability of several nutrients such as N, P, Ca, Mn, Bo and Zn decreased [36]. Decreased availability of some of these nutrients may cause plant metabolism to be not optimal and ends up in decreased plant productivity.

Root Anatomy

Table 4 shows the anatomical parameters measured at the roots of *T. erecta* L. The 50% treatment had the largest root diameter significantly different from other treatments, while the 25% treatment had the smallest root diameter. In general, there appears to be an increase in root diameter up to 75% group. This increase in diameter is likely due to Al which induces radial swelling in the roots [14]. The small root diameter of the 100% treatment plant may be unable to tolerate the highest concentration of waste, where cell growth has been stunted from the beginning due to the damage of the cortex cells by Al [14].

Besides Al, it is estimated that Cr also influences decreasing root diameter due to its high accumulation in a waste concentration of 100%. Symptoms of Cr toxicity include inhibition of root growth caused by a disruption in cell division and cell elongation or cell cycle extension in roots [32].

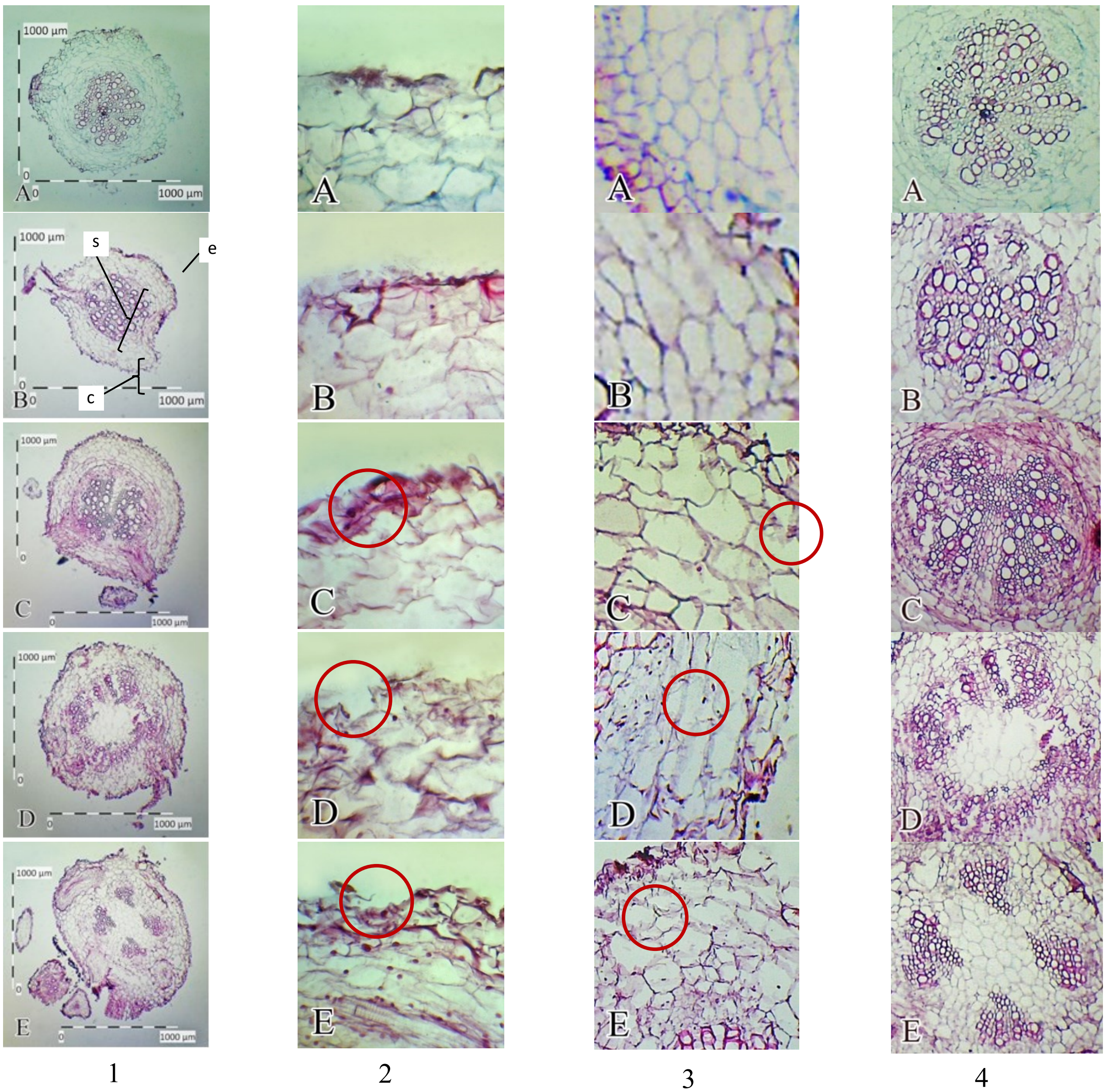

FIGURE 2. Transverse section of marigold plant root (1), root epidermal layer (2), root cortex (3) and root stele (4) after 25 days after being treated with batik wastewater with different concentrations A (0%); B (25%); C (50%); D (75%); E (100%). (**O:** damaged epidermis and cortex, e: epidermis, s: stele, c: cortex)

The epidermis is the outermost part of the root and is composed of one layer of cells that are tightly arranged. In some anatomical results obtained, the marigold root epidermis has been damaged both in the control plants and plants treated with waste due to secondary growth occurred in the root. No measurements can be made, but at certain sides there are remained layers of the epidermis. The complete epidermal condition is shown in Figure 2.

Epidermis found in control plants is rectangular in shape with intact and neat clear cell walls, while in 25% plants there are thin layers around the epidermis that may occur due to tearing of the cell wall. At the root of 50-100% plants, the shape of the epidermis cells became irregular and cell walls were torn in several places. Jones, *et al*. [14] revealed that damage of several parts of the epidermis in root was caused by the tearing of epidermis and cortex. Al induces hardening of epidermis and cortex cell walls by replacing Ca^{2+} in the cell wall and stimulating the production of cellulose , while the portion of the stele continues to enlarge. It ultimately results in the tearing of the epidermis and cortex cells.

TABLE 4. The Root Anatomical Parameters of *Tagetes erecta* L. after Being Treated with Batik Wastewater at Different Concentrations

Wastewater concentration	Root diameter (μm)	Cortex thickness (μm)	Stele diameter (μm)	Trachea diameter (μm)
0% (control)	934.95 ± 88.07^{ab}	201.25 ± 24.23^{a}	535.81 ± 7.95^{ab}	33.75 ± 4.03^{b}
25%	847.02 ± 31.52^{a}	174.18 ± 2.84^{a}	475.77 ± 17.21^{a}	35.08 ± 3.28^{b}
50%	1179.88 ± 7.73^{b}	201.7 ± 10.61^{a}	754.26 ± 14.12^{c}	41.53 ± 3.10^{c}
75%	968.35 ± 214.36^{ab}	201.53 ± 32.97^{a}	563.32± 57.54^{b}	23.94± 1.37^{a}
100%	870.82 ± 203.95^{a}	198.91 ± 18.14^{a}	492.7 ± 80.58^{ab}	22.21 ± 1.97^{a}

Numbers followed by the same letters in the same column have no significantly difference from the Duncan test with a confidence level of 95%.

Inward of the epidermis layer is cortex consisting of parenchymal cells. Cortex consists of several layers of cells. Based on the anatomical results obtained in Table 4, it is known that the 50% treatment has averagely the thickest cortical layer, while the 100% treatment has the thinnest. The cortex thickness difference between treatments is not significant, but the appearance of the cortex looked different. The greater the concentration of waste the more damaged the cells that form the root cortex. Cortex cells in control plants are polygonal, meanwhile in treated plants (75-100%) the shape of the cells are elongated or tend to be irregularly arranged. The cells in Figure 4 (D and E) look not fresh.

Jones, *et al*. [14] revealed that exposure to Al can cause cell walls and cell membranes to become rigid. This condition triggers an oxidative explosion (apoplastic oxidative burst). In certain cases, apoptosis triggered by Al is also associated with interference in Ca^{2+} regulation in mitochondria and cytochrome c which then bound to Apaf-1 and activates the caspase enzyme that triggers apoptosis (Savory, *et al.*, 2001). Damage to cortex cells in the roots of 50% to 100% concentration (Figure 10) is likely caused by oxidative explosion and apoptosis, causing a decrease in thickness of the root cortex layer. Cortex cells appeared to be enlarged up to 50% treatment before being damaged and torn at 75-100% treatment. Cortex cell enlargement is likely due to the induction of vacuole enlargement by Al. Enlargement of vacuoles is important for plants to store the excess of Al and adapt to stress [37].

Stem Anatomy

Based on the results obtained in Table 5 it is known that an increase in the diameter of stem occurred in control up to the treatment of 50% plants, then it decreased in treatment of 75% and 100%. Increased stem diameter is likely due to the enlargement of parenchymal cells in response to Al toxicity[10]. The decrease in stem diameter at concentration of 75-100% correlates with a decrease in canopy biomass which also has the lowest weight at the same concentration.

TABLE 5. The Stem Anatomical Parameters of *Tagetes. erecta* L. after Being Treated with Batik Wastewater at Different Concentrations

Waste concentration	Shoot diameter (μm)	Epidermal layer thickness (μm)	Vascular bundle thickness (μm)	Cortex (ridge) thickness (μm)	Cortex (non-ridge) thickness (μm)
0% (control)	7174.16 ± 195.08^{c}	20.69 ± 1.73^{c}	487.41 ± 1.73^{b}	224.33 ± 3.40^{c}	146.56 ± 14.56^{c}
25%	6259.33 ± 209.08^{bc}	19.03 ± 1.00^{bc}	404.1 ± 1.00^{ab}	207.43 ± 1.37^{bc}	115.33 ± 5.1^{b}
50%	8712.33 ± 835.66^{b}	17.42 ± 1.00^{b}	441.28 ± 1.00^{b}	188.66 ± 5.25^{bc}	69.96 ± 14.01^{a}
75%	5357.16 ± 271.68^{b}	16.74 ± 0.93^{b}	349.79 ± 0.93^{a}	142.9 ± 19.6^{a}	60.03 ± 8.49^{a}
100%	5256.33 ± 1007.02^{a}	14.36 ± 1.20^{a}	325 ± 1.20^{a}	175.93 ± 49.42^{ab}	52.76 ± 6.36^{a}

Numbers followed by the same letters in the same coloumn have no significantly difference from the Duncan test with a confidence level of 95%.

Lidon, *et al*. [21] revealed that in addition to the deteriorating root conditions in plants with high concentration of waste treatment, the reduction in stem diameter can also be caused by disruption of enzymatic activity that plays a role in growth such as photosynthesis and respiration, thus the more plant's growth is hampered due to high concentration of waste, the smaller the diameter of the stem produced. Observed anatomical structures of the stem are the epidermis, cortex, beam transport and pith (Figure 3).

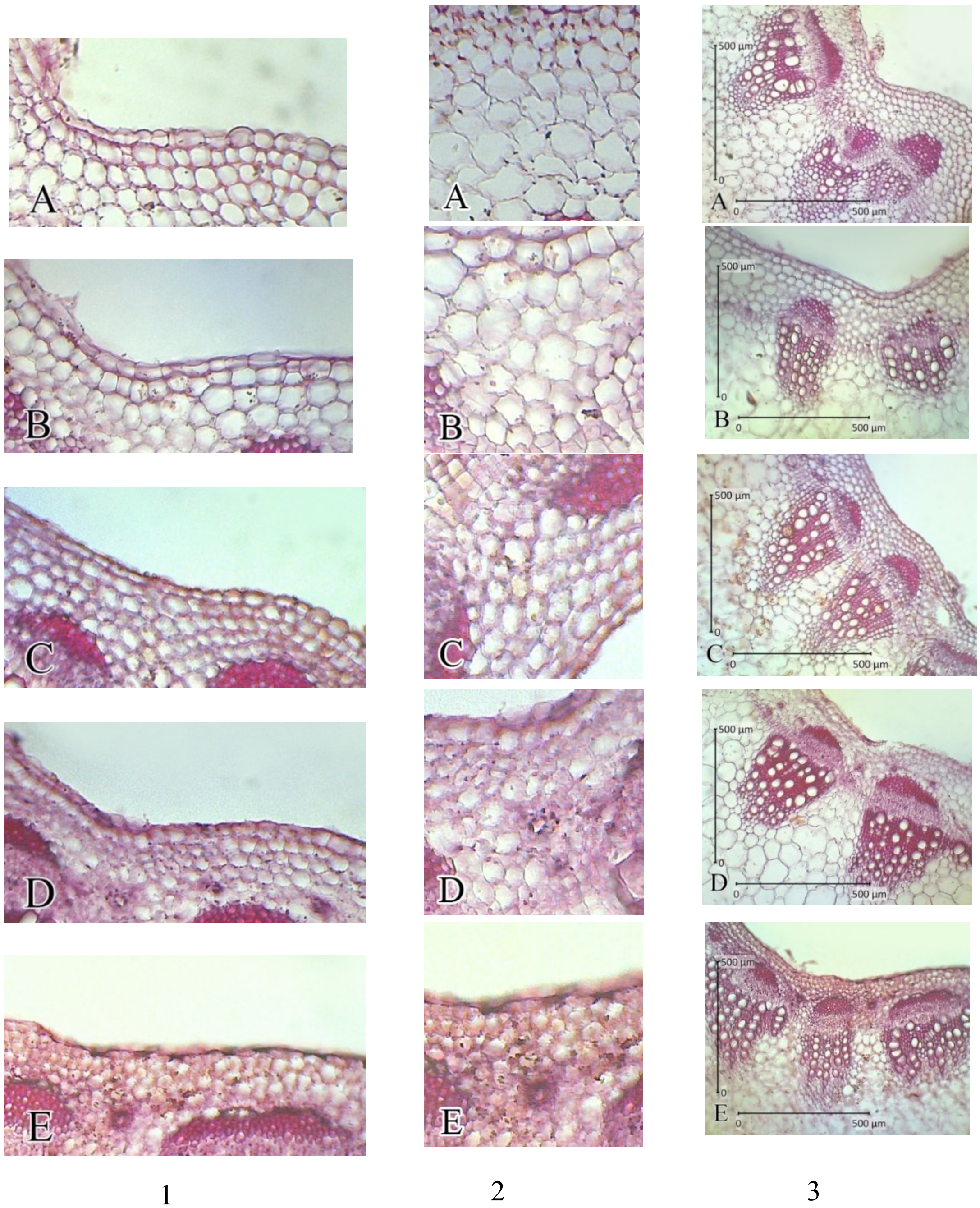

FIGURE 3. Marigold (*T.erecta* L.) stem transverse section (1), stem epidermal layer (2) and stem vascular bundle (3) after being treated with watering of batik staining liquid waste with different concentrations, (A (0%); B (25%); C (50%); D (75%); E (100%)

Stem epidermis thickness was reduced after exposure to batik liquid waste. Control plants have the thickest epidermal layer of the stem, while 100% treatment has the thinnest epidermal layer (Table 5). The reduction of epidermal cells is likely correlated with decreased elasticity of cell walls caused by Al, where the cell walls are getting stiffer, making the cells unable to develop properly. The higher the concentration of waste given, the smaller the epidermal cells of stem produced, as well as the thickening of the outer walls of epidermal cells. The thickening of the outer walls of epidermal cells is likely formed by the cuticle layer.

Konarska [17] stated that under Al stress, epidermal tissue has a thick external wall resulting from the formation of a thick cuticle layer. Al stress is often accompanied by difficulty in water intake so that plants adapted by forming a thick layer of the cuticle to limit transpiration. Besides, the thickening of the external cell wall can also be caused by the accumulation of aluminum deposits in it. Epidermal cells in the control group appear to be more developed, while in the treatment of 75-100% the epidermal cells appear to be more shrinking. This condition is likely to correlate with a decrease in the ability of the root to absorb water, resulting in low turgor pressure of the plant's constituent cells.

There was a decrease in the thickness of the stem cortex in both the ridge part and non-ridge part as an increasing concentration of the given waste (Table 5). The control group had the thickest cortex layer of the stem both in the ridge and non-ridge part, while the 100% treatment had the thinnest cortex layer. The stem cortical cells in the control group and 25% treatment are polygonal, but in 50-100% treatment the cortical cell's shape became be irregularly rounded and the size gets even smaller (Figure 3.2). Just as the cause of shrinking stem epidermal cells, the cause of shrinking stem cortex cells is probably a decrease in cell elasticity due to the presence of Al in toxic concentrations, so that the cell wall cannot be stretched optimally and the cortex layer formed becomes thinner than the control plants due to the smaller size of its constituent cells.

The thickness of stem vascular bundle decreases with increasing concentration of waste, and if observed from its constituent cells, the higher the concentration of waste, the smaller the size of the tracheal cells formed (Figure 3.3). Gomes *et al*. [10] revealed that the reduction in the thickness of the vascular bundle is likely a response to Cr toxicity to plants which serves to inhibit Cr translocation into photosynthetic tissue. It is also possible to be caused by drought stress due to Al toxicity. Water absorbed from the soil by the roots is transported through the xylem tissue to the leaves. When transpiration occurs, water is not being taken by the roots, but rather moves passively in response to the potential gradients of water formed by the transition. Heavy metal stresses affect the efficiency of water flow by decreasing the rate of transpiration and or through changes in stomatal resistance in leaves [1]. Plants defend themselves from water shortages by narrowing the vascular bundle because the wider vascular bundle has more permeable membranes so they are more susceptible to drought stress conditions [7].

CONCLUSION

Based on the research it can be concluded that, on the anatomy of marigold root (*T.erecta* L.), giving batik dyeing wastewater at a concentration of 75% and 100% reduced root diameter, cortical thickness, diameter of stele and tracheal diameter, while concentration of 50% increased all of these parameters optimally, but the condition of the epidermis and the root cortex began to damage at this concentration. In stem, the higher concentration of waste decreased the diameter of the stem, thickness of the epidermal cell, thickness of the cortical cell and size of the vascular bundle. The growth of *T.erecta* L.was increasingly inhibited along with the provision of waste with higher concentration, indicated by decreases in plant height, leaf size, root volume and length, plant biomass, chlorosis and smaller leaf size.

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