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Impact of heavily polluted air and acid rain on mulberry silkworm and cocoon production of *Bombyx mori* Linn

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Abstract
The silk is the protein polymer spun into fibers by the silkworms (*Bombyx mori* L). It is a textile fiber highly appreciated for its outstanding properties (handle, luster, dye ability, comfort). In addition, silk has been recently investigated as a starting material for nontextile applications. In order to assess the effect of air pollution on the yield and quality of silk fibers, two locations of Indian locality had been chosen on the basis of the ambient air quality for rearing of mulberry silkworm, *Bombyx mori* L. Air pollution may be indicated by the higher acid rain. Air pollution caused by the burning of fossil fuels increase emissions of SO_2, NO_2, and CO_2 which can ultimately lead to acid rain. Exposure of acid rain may affect the cultivation of mulberry and the quality of silkworm cocoons. The aim of this research was to determine the effect of mulberry plants that have been exposed to acid rain to the percentage of cocoon defects and type of defects in multivoltine races for tropical climates like C-nichi and Hosa Mysore races of *Bombyx mori* L. Acid rain that to apply on the mulberry plant for 5 weeks was a modification of acid rain that occurred in tropical area. A total of 400 individuals of the newly hatched silkworm was used in earlier research. Larvae fed by mulberry leaves that had been treated with artificial rain water in different levels of pH, i.e., pH's of 7.0 (well water as control), 6.0 (normal rain), and 5.0 (acid rain). Cocoon defects and type of cocoon defect were observed. Data were analyzed using multivariate ANOVA test. The results showed that the treatments had a significant effect on the defective cocoon percentage. Treatment of pH 7.0, 6.0, 5.0 had defective cocoons of 20%, 13%, 10%, respectively in race C-nichi 18% and 15% respectively in race Hosa Mysore. Meanwhile, cocoon defect types in race C-nichi and Hosa Mysore were outer defects consisting of printed, outside stained, malformed, very small, double cocoons, and inner defect consist of thin end and inside stained and luster of cocoons. As a whole, acid rain treatments to defective cocoon of *Bombyx mori* L. in C-nichi and Hosa Mysore races showed significant effect to defective cocoons.

Keywords: Heavily polluted air, acid rain, mulberry silkworm, cocoon production, *Bombyx mori* Linn

Introduction
The main air pollutants are represented by gases forms, particles in suspension, different ionizing radiation and noise. The gases forms are: oxidized and reduced forms of carbon (CO_2, CO, CH_4), of nitrogen (NO_2, NO, N_2O_4, NH_3, NH^{4+}), SO_2, O_3, C_6H_6 vapors, Hg, volatile phenols, Cl_2, etc. The particulate forms are: PM10 and PM2.5 particulate matter, heavy metals with toxic effect (Pb, Ni, Cd, As), polycyclic aromatic hydrocarbons PAHs, etc. Atmospheric pollutants have a negative effect on the mulberry plants; they can have direct toxic effects, or indirectly by changing soil pH followed by solubilization of toxic salts of metals like aluminum. The particulate matters have a negative mechanical effect. They cover the leaf blade reducing light penetration and blocking the opening of stomata. These impediments influence strongly the process of photosynthesis, which rate declines sharply. Also, the leaves of the trees have an important role in retention of the particulate matters; they are mostly affected when the wet and dry atmospheric deposition increase. The vegetation plays an important positive role in atmospheric purification and air pollutant reduction. The primary producers represented by plants are an important component in biogeochemical cycles. The vegetation made exchanges with a part of the atmospheric gases by photosynthesis, respiration processes, and the final stage of litter decomposition which mineralization. The plants play an important role in reducing atmospheric CO_2 content, by photosynthesis. This reduction of atmospheric CO_2 content has an important role in reducing of greenhouse gases, participating in reducing the greenhouse effect and its consequences on climatic changes.

The carbon stored in plants is the result of balance between carbon fixed by photosynthesis and carbon released in the atmosphere by respiration. As the structure of the vegetation is more complex, the carbon stock in plant biomass is higher and the period of storage is longer. The most efficient type of vegetation in storing carbon in terms of carbon stored in plants alive is the temperate-continental forest; and in terms of carbon stored in dead organic matter are peat lands. Trees have also been planted to reduce the intensity of ionizing radiation and noise in different urban and industrial areas. The existence of vegetation in an area creates a microclimate where the temperature differentials between day and night are buffered. This prevents the occurrence of warmer temperatures, which stimulate the production of volatile pollutants into the atmosphere. Environmental pollution is any discharge of material or energy into water, land, or air that causes or may cause acute (short-term) or chronic (long-term) detriment to the Earth's ecological balance or that lowers the quality of life. Pollutants may cause primary damage, with direct identifiable impact on the environment, or secondary damage in the form of minor perturbations in the delicate balance of the biological food web that are detectable only over long time periods. Air pollution is the process which the substances and the energy forms are not present in the normal atmospheric composition, reach the atmosphere, or are present but in much lower concentrations. Air pollution is the introduction of chemicals, particulate matter, or biological materials that cause harm or discomfort to humans or other living organisms, or cause damage to the natural environment or built environment, into the atmosphere. The global pollution is the result of cumulative effects of various sources, located on the entire surface of the globe, manifested by global effects: the stratospheric ozone depletion; greenhouse effect - emission of greenhouse gases (CO_2, methane, CFCs, etc.); formation of aerosols (pollutant clouds which suspended particles and chemical compounds). The regional pollution is in part the result of local air pollution--including that produced by individual sources, such as automobiles - that has spread out to encompass areas of many thousands of square kilometers. Meteorological conditions like temperature, photoperiod & humidity and landforms can greatly influence air-pollution concentrations at any given place, especially locally and regionally. For example, cities located in bowls or valleys over which atmospheric inversions form and act as imperfect lids are especially likely to suffer from incidences of severe smog. Oxides of sulfur and nitrogen carried long distances by the atmosphere and then precipitated in solution as acid rain, can cause serious damage to vegetation, waterways, and buildings. The local pollutants (smog) can be loosely defined as a multi-source, widespread air pollution that occurs in the air of cities. Smog, a contraction of the words smokes and fog, has been caused throughout recorded history, by water condensed on smoke particles, usually by burning coal. In terms of the effects of pollutants can be acidifying agents - Sulphur dioxide (SO_2), nitrogen oxides (NOx) ammonia (NH_3) fluoride and Cl_2, hydrogen chloride (HCl) - and oxidizing agents - carbon monoxide (CO), PAN (peroxyacetylnitrate-$CH_3CO.O_2.NO_2$), ozone (O_3). Sources of pollutants

Materials and Methods

Acid Rain Preparation: Artificial rains were arranged by adding demineralized water with various minerals found in the polluted rain in Dewari, Kachan, Myorpoor, Block DC Sonbhadra, India U.P. The acid rain was made by adding a solution of artificial rain and sulfuric acid until the pH reached 5.0.

Method: Research was conducted in Malang, Indonesia. Mulberry with the condition 4 weeks after pruning were watered by artificial acid rain for 5 weeks. Water volume of 500 mls/ polybag watered Mulberry 18 times. All mulberry plants were under a plastic sheet covering. Silkworm races that used in this research were C-nichi and Hosa mysore races. The C-nichi race was well adapted to the environment [15]. Meanwhile, Hosa Mysore was not well adapted, but it had a greater cocoon [16]. Newly hatched larvae of silkworms were reared using guidelines of Dewari, Kachan, Myorpur, Block DC Sonbhadra, India U.P. There were 6 combinations of treatments with four replications, each consisted of 30 larvae. There were 400 individuals' larvae used in early research. The entirely of the cocoon crop obtained was calculated in the percentage of defective cocoon and the types of cocoon defects. Percentage of defective cocoon was calculated as defective cocoon / total cocoon X 100. Data were analyzed using multivariate ANOVA.

Result and Discussion

The quality of fresh cocoon based on visual test and can be determined easily first by calculating the percentage of normal cocoons and defect cocoons [17]. Defective cocoon cannot be avoided, but it can be minimized. Naturally, silkworms have gregarious behavior in their activity, including at cocooning. So, Seri frame was filled with the fifth instar as many as 10 larvae each row. The behavior of the larvae weaving cocoons in a position very close to another causes double cocoon, which is a type of defect cocoon. Double cocoon is caused by a crowded mounting condition, high temperature and high humidity, and the mutation of the silkworm species.

Percentage of defective cocoon caused by acid rain Treatments Defective Cocoons (%) C-nichi race Well water pH-7.0 9.28 notched, 1.03 Printed &1.04 malform artificial rain pH-6.0 7.83 notched, 1.74 printed & 0 malform and artificial acid rain pH-5.0 4.35 notched, 1.74 printed & 1.74 malform of cocoon. In this study, the determination of the cocoon defects used a full population of the entire amount of cocoon produced. In this study, there were some malformed types of cocoons. These cocoons would not produce normal fiber and not an original luster, color of cocoons. In addition, concerning outer defects, defective cocoons in this study were also seen from the number of inside stained cocoon. For a common researcher, inner defects are rarely considered in measuring the quality of the cocoon, even though the percentage is large. Usually, inner defects are seen in the measuring process by the length of the fiber and production of cocoon. Inside stained cocoon as an inner defect in which the pupa is dead and sticks to the inside shell of the cocoon. These cocoons are difficult to process and will result in silk, which is dull in color and cocoon productivity.

Table 1: Percentage of defective cocoons of *Bombyx mori* L. C-nichi race Treatments

Treatments	Outer defective cocoon (%)				Very small	Double	Total	Inner defect	cocoons (%)	Total
	Notch ed	Printed	Outside stained	Mal formed				Thin end	Inside stained	
Well water pH-7	9.28	1.03	0	1.04	0	0	11.34	10	0	10
Artificial Rain pH-6	7.83	1.74	0	0	0.87	0	10.44	2.5	0	25
Artificial rain pH-5	4.35	1.74	0.87	1.74	0	0	8.7	2.5	0	2.5

Hosa Mysore race of cocoon impact of treatment percentage as well water pH-7.0 0.94 notched, 3.78 printed & 0.0 malform, Artificial rain pH-6.0 0.0 notched, 1.67 printed & 0.83 malform and Artificial acid rain pH-5.0, 0.45 notched, 0.86 printed & 0.0 malform of cocoon. Besides cocoon weight, cocoon quality, color luster parameters that are commonly used to determine the grade is the number of cocoon quality defects. Defective cocoons in Hosa Mysore showed many notched cocoons, printed cocoons, outside and inside stained cocoons. Printed cocoons had thin fibers and mutually attached to each other, so they easily break when reeled. Stained cocoons caused low quality of yarn, due to the yarn color was not uniform. Results of analysis of variance (ANOVA) showed that acid rain watering to Mulberry had significant effects in defective cocoon. Thus, it can be stated that the watering treatment of acid rain and the type of race silkworm cocoon affect the percentage of defective cocoon. Cocoon quality is very dependent on spinning condition and reeling of silk.

Table 2: Percentage of defective cocoons of *Bombyx mori* L. Hosa mysore race

Treatments	Outer defective Cocoon (%)				Very small	Double	Total	Inner defect	Cocoons (%)	Total
	Notch ed	Printed	Outside stained	Mal formed				Thin end	Inside stained	
Well water pH-7	0.94	3.78	0.94	0	0.94	1.89	8.49	10	0	10
Artificial Rain pH-6	0	1.67	2.50	0.83	0	0	5.00	7.5	2.5	10
Artificial rain pH-5	0.45	0.86	3.45	0	0	0	7.76	2.5	5.0	7.50

Environmental conditions should be quiet involve in sericulture. There can be no loud noises that would cause caterpillars to stop spinning, such as the presence of lightning. When the environment is disturbed, events that arise are thin fibers, or broken, or are not homogeneous in diameter. In addition, silkworm should not be disturbed, for example by moving the Seri frame. Temperature and humidity conditions should be considered when spinning. The ideal condition for maximum productivity is 23-30 °C in temperature and 60-90% in humidity [10]. In this research, the room temperature was 20-35 °C and humidity was 70-85% in the dry season. However, breeds in the dry season are more difficult than rainy season. Rearing of multivoltine race of C-nichi & Hosa Mysore during summer is very difficult with frequent crop losses, because had high temperature and high humidity. One location was considered polluted as about six Polynuclear Aromatic Hydrocarbons (PAH) have been identified and the concentrations of them constituted to be as high as 8.0 µg /m3 apart from heavy particulate matter in the ambient air sample of that area, while the other location (Dewari, Kachan, Myorpur, Block DC Sonbhadra, India U.P.) was the control. Different biological indicators for the silkworms as well as yield and quality of silk fibers between the two sites were considered extensively and it was found that the overall condition of the silkworms as well as the yield and quality of silk is better in less polluted areas.

Table 3: Effect of air pollution during late-age rearing traits of silkworm

Traits	Air Polluted Temperature (°C)				Control 26(°C)
	20±2(°C)	25±2(°C)	30±2(°C)	35±2(°C)	
Pupation (%)	75.99	95.02	90.77	86.77	96.22
Cocoon weight (g)	1.33	1.99	1.88	1.77	2.01
Shell weight (g)	0.39	0.47	0.45	0.41	0.49

Air Polluted Factors	Incubation	Ist instar	IInd instar	IIIrd Instar	IV instar	Vth instar	Spinning	Cocoon Preservation
Temperature	20±2(°C)	25±2(°C)	30±2(°C)	35±2(°C)	25±2(°C)	26±2(°C)	28±2(°C)	25±2(°C)
Relative Humidity	70±5%	75±5%	80±5%	85±5%	80±5%	65±5%	70±5%	80±5%

Air pollution comes from natural and anthropic sources; these sources generate pollutants with different effects at the global level or on individuals of plants and animals. Natural processes that affect air quality include volcanoes, which produce sulfur, chlorine, and ash particulates. Wildfires produce smoke and carbon monoxide. Cattle and other animals emit methane as part of their digestive process. Even pine trees emit volatile organic compounds (VOCs). Many forms of air pollution are human-made. Industrial plants, power plants and vehicles with internal combustion engines produce nitrogen oxides, VOCs, carbon monoxide, carbon dioxide, sulfur dioxide and particulates. In most mega-cities, cars are the main source of these pollutants. Stoves, incinerators, and farmers burning their crop waste produce carbon monoxide, carbon dioxide, as well as particulates. Other human-made sources include aerosol sprays and leaky refrigerators, as well as fumes from paint, varnish, and other solvents. One important thing to remember about air pollution is that it doesn't say in one place. Winds and weather play an important part in transport of pollution locally, regionally, and even around the world, where it affects everything, it comes in contact with. The major anthropic sources of air pollution are: industry and conventional energies (the mining industry, the energy industry based on fossil fuels - coal, oil, natural gas, central heating, chemical and metallurgical industry, engineering internal combustion machinery industry,

industrial waste, noises, etc.); agriculture (the vegetation fire, denitrification in soils excessively fertilized, paddy field, intensive husbandry, deforestation, etc.) transportation (motor vehicle pollution, noises, etc.). and urbanization (sewage plans, authorized landfill site, etc.) The most important atmospheric pollutants. The air pollutants are represented by gases forms, particles in suspension, different ionizing radiation and noise.

Major gaseous pollutants

Fluoride is released into the air in large quantities by aluminum reduction plants, phosphate processors, steel mills, coal burning operations, brick and tile manufacturers, and various less significant sources [1]. It can cause adverse effects when ingested by domestic animals or absorbed by plants. There are also reports that fluoride air pollution can adversely affect human health, though these are less well documented than those concerning sensitive animals and plants. Fluorides are released into the air in both a gaseous state (as hydrogen fluoride and silicon tetra-fluoride) and in solid particles. The particles fall on, and the gases are absorbed by, vegetation near the polluting industry. If this vegetation includes forage crops which are fed to cattle, sheep, horses, or pigs, serious problems may ensue, since these animals, particularly the cattle are vulnerable to fluoride [2]. In fact, according to the U.S. Ninety-six percent of the ingested fluoride that accumulates in the bodies of animals is incorporated into the crystal structure of bone and tooth mineral [3, 4]. When fluoride is ingested with food or water, most of that which is not deposited in the bones, teeth, and other calcified tissue is excreted in the urine within hours of ingestion [5]. Thus, it is not surprising that fluoride mainly affects the bones and teeth. Teeth are more markedly affected by ingested fluoride than are bones, but their high sensitivity is limited to the period of their formation. Thus, a cow that has not been exposed to excessive fluoride before the age of two and one-half to three years will not develop the severe dental lesions which would occur in the same animal exposed at a younger age [6]. The developing tooth exposed to small amounts of fluoride may experience color variations ("mottling") that have little or no effect on the animal's ability to eat. Higher levels of fluoride result in more serious dental abnormalities, ranging from small, brittle, chalky areas on the tooth surface to pitting of enamel and easily eroded teeth. Even more serious effects, including severe pain and the wearing down of the tooth right to the gum, can prevent the cattle from drinking cold water or eating. Localized or generalized enlargement of certain bones in the legs (metacarpals and metatarsals) and the lower jaw (mandible) of cattle are common symptoms of excessive fluoride ingestion [7]. As highly abnormal bone tissue replaces normal bone, [8] overall enlargement occurs, and the normally smooth bone surfaces take on a chalky, white, irregular appearance. Hard ground can cause fluorotic hoof (pedal) bones to fracture, resulting in severe lameness [6]. Cattle with advanced fluorosis may also be crippled by mineralization of ligaments, tendons, and the structures surrounding the joints [9]. Enlargement of the joints themselves may also contribute to lameness. Fluoride-induced tooth destruction, lameness, and stiff joints affect the animal's ability to stand, eat, and graze, and all tend to lower the milk yield of dairy cattle or the weight of beef cattle [12]. Chlorine, although chlorine concentrations change very rapidly in the atmosphere due to atmospheric chemistry and light rain can remove all the chlorine from the air in a very short time, chlorine injury can occur in plants near the source of pollution. The impact of chlorine pollution increases in bright sunlight and decreases in drought and low temperature.

Many particulate and gaseous fluorides are produced when ores containing fluorine are processed and used in industries. Common gaseous fluoride pollutants are HF, SiF_6, CF_4 and F_2. Particulate fluoride pollutants include Ca_3AlF_6 (Cryolite), CaF_2, NH_3F, AlF_6, CaSiF, NaF and Na_2SiF_6. Aerosols are often formed from NaF, $NaAlF_6$ and AlF_6. Chief sources of fluoride pollutants are brickworks, aluminum factories, glassworks, steelworks, ceramic [11] factories, phosphate fertilizer plants and uranium smelters. Some fluorine pollution also occurs during combustion of coal. Most injurious fluoride pollutant is gaseous hydrogen fluoride (HF).

Environmental context: NH_3 contributes to acid deposition (plays an important role in acidification) and eutrophication. The subsequent impacts of acid deposition can be significant, including adverse effects on aquatic ecosystems in rivers and lakes and damage to forests, crops and other vegetation. Eutrophication can lead to severe reductions in water quality with subsequent impacts, including decreased biodiversity, changes in species composition and dominance, and toxicity effects [13]. NH_3 also contributes to the formation of secondary particulate aerosols, an important air pollutant due to its adverse impacts on human health.

VOC (Volatile Organic Compounds) VOC can be a range of different contaminants, such as carbohydrates, organic compounds and solvents. These compounds usually derive from petrol a nd gasoline reservoirs, industrial processes and fuel combustion, paint and cleanser use, or agricultural activities. VOC play an important role in ozone shaping in the lower atmospheric layer, the main cause of smog. VOC can cause various health effects, depending on the kind of compounds that are present and their concentrations. The effects can vary from smell nuisance to decreases in lung capacity, and even cancer [14].

Conclusion

The present investigation concluded that the atmospheric composition, falling in the atmosphere can be considered air pollutants. Some substances that are normally present in the atmosphere in a certain concentration can be considerate pollutants because their concentration is much higher than usual concentration of different races of multivoltine mulberry silkworm. Also, certain substances that are normally present in certain layers of the atmosphere (e.g., ozone in the stratosphere), once arrived in the troposphere is pollutant. Some gases, such as oxides of nitrogen may have beneficial effects on vegetation, after hydration may affect the leaf fertilizer. The air pollutants factors can be chemical (chemicals), mechanics (particles in suspension) physical (ionizing radiation) and acoustic (noise). Pollutants describe a global circuit; they are produced by different sources, are transported and transformed into an atmosphere, some of them being removed, another part is reaching the earth, having different effects on different business of ecosystems and mulberry plant which the main feeding sources of silkworm life. Currently, air, acid rain, pollution has become a larger area, sometimes to disperse across multiple continents. Our findings demonstrate environmentally & rearing technology induced quality parameters that must not be ignored when analyzing and deploying silk cocoon, silkworm

growth, silk filaments or silk derived bio-polymers and mulberry leaf production.

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