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EFFECTS OF PRETANNING EFFLUENT ORIGINATED FROM LEATHER INDUSTRY ON THE GERMINATION OF RICE SEEDS

The effects of pretanning effluent obtained from leather processing on the germination and seedling growth of rice were investigated. Undiluted pretanning effluents, and its 1:10, 1:40 and 1:80 dilutions along with tap water were used for irrigation to germinate the seeds. Some morphological characteristics were investigated. No germination occurred in the seeds irrigated with the undiluted pretanning effluent. Elemental analyses of the shoots and roots of rice seedlings were conducted. The lowest contents of C (29.7%), S (0.41%), Cr (1.2 mg/kg) and Na (2933.8 mg/kg) in shoots were detected in control group. The lowest contents of Cu (38.81 mg/kg) and Mn (2225.0 mg/kg) in roots were determined in irrigations with 1:10 dilutions.

1. INTRODUCTION

Converting of raw skins into final leather comprises pretanning, tanning, and post-tanning stages. Soaking, dehairing, liming, deliming, bating, degreasing, and pickling steps are prerequisites to remove hair, epidermal layer, flesh, fat and globular proteins. These steps are called pretanning processes. Pollutants such as NaCl, Ca(OH₂), Na₂S, detergents, ammonium compounds, and organic solvents disposed by tannery are majorly originated from pretanning processes, in which the highest amount of water is used and disposed. The pollution load (biochemical oxygen demand, chemical oxygen demand, total suspended solids, total dissolved solids, sulfur

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contents, sludge) at the pretanning process is much higher than the tanning and post-tanning processes [1]. The amount of water used in processing hides or skins varies across tanneries. Recently, the amount of effluents has notably decreased due to legal limitations on pollution and increasing interest in ecological production methods [2–4].

Effluents from leather production are discharged into environment without treatment not only in some leather manufacturing locations in Turkey [5, 6] but also across the world [7–9]. Although tannery effluents contain high levels of contaminants, which adversely affect soil and water bodies, unfortunately polluted waters are still used for irrigation in some countries. Rice (*Oryza sativa*) has a distinctive characteristics; it can germinate in water and absorb oxygen from water. Rice, a cultivated plant for centuries, is commonly used for nutritional and industrial purposes. It occupies a considerable place in daily diets of the Far Eastern and Indian people. In Turkey, 900 000 tons of rice was produced in the area of 99 400 hectares with a mean yield of 9050 kg per hectare in 2011 [10].

It is evident that many problematic contaminants in the effluents not only pollute the environment. They are discharged into but also threaten the ground and surface water, aquatic life, and soil ecosystem. In addition, it is of critical importance to determine whether products grown in polluted soils and circulated in the dietary supply chain have been contaminated or not. Many investigations have been conducted on the subjects mentioned above [11, 12].

The aim of the present study was to determine the germination and seedling growth of rice plants germinated with the use of pretanning effluent (1:0) and its diluted (1:10, 1:40 and 1:80) concentrations. Moreover, the changes of some elemental contents in roots and shoots of seedlings were studied. This study will provide a basic knowledge for future *in-situ* research on rice production with the contaminated soils by tannery effluents.

2. MATERIALS AND METHODS

Domestic sheep skins were used to obtain pretanning effluent. These skins were processed until the tanning stage in accordance with fundamental principles for garment leather production [13] and with a general leather processing recipe (Table 1).

Effluents, obtained separately from every pretanning process, were brought together in a dark container to form the undiluted effluent (1:0) (Fig. 1). Then, this effluent was diluted in the ratio 1:10, 1:40 and 1:80 w/w (undiluted effluent: tap water). In order to determine the effects of pretanning effluents on germination and seedling growth of rice, tap water (for control purposes) and undiluted effluent (1:0) were used along with diluted effluents (1:10, 1:40 and 1:80) as irrigation water.

Table 1

Treatment procedure of wet-salted sheep skins

| Process | [%] | Chemicals | Temp. [°C] | Time [min] | pH | MA ¹ (rpm) |
|--|--|---|------------|------------|---------|-----------------------|
| Percent based on raw skin weight | | | | | | |
| Presoaking | 500 | water | 20 | 240 | 7.0–7.5 | 0 |
| Drain | | | | | | |
| Main soaking | 500 | water | 20 | | | |
| | 0.5 | non-ionic emulsifier | | | | |
| | 0.5 | bactericide | | 30 | 7.0–7.5 | |
| | (run on automatic – run 10 min/stop 50 min for 18 h in a paddle) | | | | | |
| Drain, prefleshing | | | | | | |
| Painting | 15 °Bé Na ₂ S–25 °Bé Ca(OH) ₂ solution applied onto the flesh side of the skin | | | | | |
| Unhairing | | | | | | |
| Liming | 400 | water | 20 | | | |
| | 2 | Na ₂ S | | | | |
| | 4 | Ca(OH) ₂ | | 30 | 12 | 4 |
| | (run on automatic – run 3 min/stop 57 min for 24 h) | | | | | |
| Drain, fleshing, trimming, weighing (recorded as pelt weight, % based on pelt weight) | | | | | | |
| Deliming–bating | 300 | water | 35 | | | |
| | 1.5 | ammonium sulfate | | 30 | 8.3–8.5 | 12 |
| Add | 1 | proteolytic enzyme | | 60 | | 12 |
| Wash | 300 | | 20 | 10 | | 12 |
| Drain | | | | | | |
| Degreasing | 100 | water | 35 | | 5.5 | |
| | 5 | degreasing agent | | 90 | | 12 |
| Washing | 100 | water | 35 | | | |
| | 2 | NaCl | | 30 | | 12 |
| Washing | 100 | water | 35 | | | |
| | 2 | NaCl | | 30 | | 12 |
| Washing | 100 | water | 35 | 30 | | 12 |
| Pickling | 150 | water | 20 | | | |
| | 5 | NaCl | | 10 | | |
| Add | 0.5 | HCOOH (diluted 1:10) | | 30 | | 12 |
| Add | 1 | H ₂ SO ₄ (diluted 1:10) | | 90 | 2.9-3.1 | 12 |

¹MA – Mechanical action of leather processing drum.

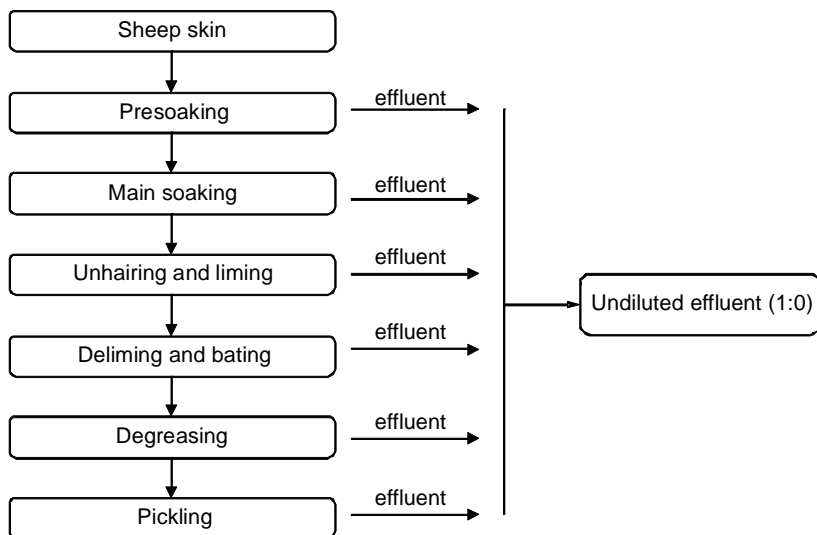


Fig. 1. Flow diagram of pretanning processes where undiluted effluents (1:0) were obtained

Table 2

Physicochemical characteristics of the pretanning effluents and tap water¹

| Parameter | Tap water | Pretanning effluent at dilution of | | | Undiluted pretanning effluent (1:0) |
|--------------------|-----------------|------------------------------------|-------|-------|-------------------------------------|
| | | 1:80 | 1:40 | 1:10 | |
| pH | 7.25 | 8.25 | 8.68 | 11.05 | 12.26 |
| EC, mS/cm | 0.54 | 1.20 | 1.75 | 5.28 | 46.3 |
| NH ₄ -N | 0.25 | 0.32 | 0.15 | 0.60 | 4.28 |
| NO ₂ -N | 0.02 | 0.09 | 0.13 | 0.17 | 1.16 |
| NO ₃ -N | 3.12 | 6.96 | 7.89 | 30.19 | 127.0 |
| Total S | nd ² | 0.88 | 6.8 | 320 | nd ² |
| K | 1.59 | 8.28 | 18.58 | 55.76 | 760.99 |
| Ca | 39.40 | 59.32 | 60.49 | 88.48 | 261.21 |
| Mg | 2.14 | 2.73 | 3.54 | 5.98 | 13.41 |
| Na | 14.0 | 125.7 | 253.3 | 801.4 | 9466.4 |
| Cu | 0.022 | 0.200 | 0.230 | 1.151 | 4.76 |
| Cl | 31.5 | 245.5 | 538.2 | 1674 | 12638 |
| SO ₄ | 23.9 | 64.7 | 85.3 | 157.8 | 644.7 |

¹All values, except pH and EC, are in mg/dm³.

²nd – not detectable.

Physicochemical properties of the prepared irrigation waters were analyzed in the the Scientific Technology Research Centre at the Çanakkale Onsekiz Mart University (COBILTUM) and the results are given in Table 2. A thermo aquamate spectropho-

tometer (Merck Test Kit) was used for the analyses of ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen, and an ion chromatograph system (Shimadzu, Japan) was used for analysing chloride and sulfate ions. The other elements were determined by ICP-AES-Varian Liberty AX sequential. For determination of the anions and elements concentration of irrigation waters the instruments were calibrated by using 100 ppm standard anion and element solutions by serial dilutions (10, 5, 2.5, 1, 0.5, and 0.1 ppm). Every measurement was performed as triplicate, and the average values were given in Table 2.

Seeds of Osmancık variety were used as the plant material. Before sowing, the seeds were pregerminated. The soil into which the seeds were sown was analyzed in the Soil-Leaf-Water Analysis Laboratory of the Çanakkale Directorate of Agricultural Affairs. Results obtained from the analyses are provided in Table 3. The soil used was non-saline and neutral, and the organic matter content was at a medium level whereas P, K and Ca levels were very high. The study had a randomized block design with three replications. The cases where the seeds were sown were kept at room temperature. Three weeks (on the 21st day) after sowing, the seedlings were carefully uprooted and then some morphological properties such as germination percentage, root length, root weight, shoot length and shoot weight were examined.

Table 3

Physical and chemical characteristics
of the experimental soil

| | |
|-------------------|-------|
| Saturation, % | 55 |
| Salinity, dS/m | 880 |
| pH | 7.28 |
| Lime, % | 1.61 |
| Organic Matter, % | 2.44 |
| P, mg/kg | 65 |
| K, mg/kg | 209 |
| Ca, mg/kg | 6864 |
| Mg, mg/kg | 1401 |
| Na, mg/kg | 1.00 |
| Fe, mg/kg | 18.77 |
| Cu, mg/kg | 1.70 |
| Mn, mg/kg | 34.32 |
| Zn, mg/kg | 2.48 |

For the laboratory analysis, 0.5 g of plant materials (shoots and roots) were digested in 9 cm³ of concentrated nitric acid and 3 cm³ of hydrofluoric acid for 15 min using microwave heating system at 180±5 °C. After cooling, the contents were filtered, centrifuged and diluted. And then Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na and Zn contents of the roots and

the shoots of rice were determined using ICP-AES-Varian Liberty AX sequential [14], similarly as in the case of irrigation waters. An IR detection system (Leco S-C 144 DR) was also used for the C and S elements of the shoots in the COBILTUM.

Data obtained were tested by statistical analyses of variance [15] and means were compared by the LSD test ($p < 0.05$).

3. RESULTS AND DISCUSSION

Data on the germination and seedling growth of rice irrigated with 1:10, 1:40 and 1:80 dilutions are given in Table 4. Due to high pH (12.26) and high values of other parameters of the undiluted effluents (1:0) (Table 2), the seeds were adversely affected by the effluent and no germination occurred. Therefore, the data related with this group were excluded from the statistical analyses and were not presented in Tables 4–6.

Table 4

Seed germination and growth characteristics of rice seedlings

| Treatment | Germination [%] | Root | | Shoot | |
|--------------------------|-----------------|-------------|-------------|-------------|-------------|
| | | Length [cm] | Weight [mg] | Length [cm] | Weight [mg] |
| Tap water | 77.8 a | 5.80 a | 2.60 a | 35.7 a | 58.7 a |
| 1:80 | 65.3 b | 4.47 b | 1.87 b | 33.8 a | 47.0 b |
| 1:40 | 58.7 cb | 3.97 b | 1.55 b | 22.2 b | 38.1 c |
| 1:10 | 52.0 c | 3.17 c | 0.87 c | 6.2 c | 11.0 d |
| LSD _{0.05} | 7.57 | 0.66 | 0.33 | 3.38 | 7.34 |
| Coefficient of variation | 5.97 | 7.62 | 9.70 | 6.92 | 9.49 |

Within columns, means with different letters are statistically significant, $p < 0.05$.

In Table 4, the highest percentage (77.8%) of germination was observed from tap water treatments, while the lowest value (52%) was obtained from the 1:10 diluted effluent. The differences between the treatments were statistically significant ($p < 0.05$). There are many studies resulting in adverse effects on the germination and seedling growth of various plants irrigated with tannery effluents. Tayyar and Yapici [16] investigated the effects of pretanning effluents of hides on the germination of broad bean, lentil, and common bean, and found no germination in the undiluted effluent (1:0). Yapici and Tayyar [17] examined the effects of pretanning effluents on the germination and seedling growth of some cereals, and found significant differences between the treatments. They revealed that undiluted effluent (1:0) reduced germination. Indra and Rai Mycin [18] suggest that tannery effluents contain both valuable nutrients and contaminants such as salt and chrome which may have negative effects on the growth of crops. They investigated the effects of concentration (10, 20, 30, 40,

50 and 100%) of tannery effluents on seed germination and seedling growth of *Vigna mungo L.* The data with 10% of tannery waste water increased the germination, percentage growth, fresh and dry weight of *V. mungo L.*, while the higher concentration decreased all of the parameters.

Calheiros et al. [19] conducted research on the effects of effluents obtained from the inlets and outlets of different sections of tannery effluent treatment facilities on the germination of *Trifolium pratense*, *Typha latifolia*, and *Phragmites australis*. The researchers concluded that an increase in the level of the effluent in the irrigation water causes decrease in the germination rate. Contreras-Ramos et al. [20] claimed that tannery effluents contain toxic organic compounds that could affect the soil processes, plant growth, and pathogens along with valuable nutrient agents. They also assumed that the high level of salt concentration might inhibit the germination, which caused a decrease in the germination index.

Many researchers stated that it could be impossible for plants to grow in soils with pH of 8.5 and higher and high salinity values. When physicochemical properties of pretanning and tap water were analyzed (Table 2), pH of undiluted effluent increased to 12.26, most probably because of the increasing sodium amount that was 8.25 even in the 1:80 diluted effluent. According to the same table, salinity values incrementally increased with the higher effluent concentration that caused low percentages of rice germination (Table 4).

The highest values of root lengths and weights were obtained with tap water, while the lowest were observed with 1:10 diluted effluent. The highest shoot lengths were observed with tap water (35.7 cm) and 1:80 diluted effluent (33.8 cm), while the lowest shoot length (6.2 cm) was measured with 1:10 diluted effluent. 1:40 dilution (22.2 cm) was found to be between these extremities (Table 4). The heaviest shoot (58.7 mg) was observed in the tap water and the lightest one (11.0 mg) in the 1:10 diluted effluent.

Root growth of plants is directly and indirectly related with pH. Direct effects of high pH are regarded as the fluctuations in the inter-membranous pH values, changing electro potential levels and proton-anion co-transportation in the plasma membrane [21]. In this research, the high contents of ammonium in the undiluted effluent might have a direct adverse effect on the root growth due to toxic properties. Gupta and Sinha [22] suggested that plants can absorb from the soil and water more than they essentially need and that even a small amount of metals they need can keep them growing, but over-absorption of such metals can cause toxicity. In addition, Marschner [21] stated that high bicarbonate concentration leads to high pH values, and inhibits root growth. From this viewpoint, it is possible to understand the reason for the differences in root length and weight values given in Table 4. It can also be asserted that these inhibitive factors directly and adversely affect the shoot lengths and weights in this experiment.

Some elemental contents determined in the shoots of the seedlings irrigated with various dilutions of pretanning effluents and tap water (control) were given in Table 5. It has been proven by other authors that high levels of pH, calcium, chlorine, magnesium, sodium, and potassium both individually and complex interactions have adverse effects on the nutrient absorption capability of plants and thus on their growth. Tisdale, Nelson [23], and Bergmann [24] claimed that high lime and pH values reduce boron, copper, iron, manganese, and zinc absorption. Kacar [25] found that overabundance of calcium and magnesium results in phosphor fixation due to high pH values.

Table 5

Some elemental contents of the shoots¹

| Element | Irrigation with tap water | Irrigation with pretanning effluent at dilution | | |
|---------|---------------------------|---|---------|---------|
| | | 1:80 | 1:40 | 1:10 |
| C | 29.70 | 30.27 | 31.85 | 31.69 |
| S | 0.41 | 0.47 | 0.61 | 1.19 |
| Al | 276.3 | 302.6 | 483.4 | 1211.8 |
| Ca | 4631.3 | 5140.7 | 5832.1 | 9129.9 |
| Cr | 1.20 | 1.86 | 1.70 | 8.29 |
| Cu | 22.44 | 19.17 | 16.37 | 12.21 |
| Fe | 340.4 | 322.4 | 533.0 | 1455.3 |
| K | 20579.7 | 18123.5 | 16770.6 | 15783.3 |
| Mg | 2173.5 | 2052.4 | 2277.5 | 2718.6 |
| Mn | 1150.5 | 961.5 | 615.3 | 671.5 |
| Na | 2933.8 | 7288.6 | 6632.0 | 7160.9 |
| Zn | 87.57 | 88.85 | 71.15 | 61.04 |

¹C and S contents in %, the others in mg/kg.

Toxic effect of excessive amount of chlorine on plants has been known by scientists from the analyses of the effects of road salting. Excessive chlorine, which gives rise to chlorosis and necrotic spots on the plant's parts caused nitrogen, phosphor and potassium levels to go below normal levels but caused an increase in the calcium and magnesium concentrations [24]. Also, ammonium overabundance causes an increase of the toxicity of chlorine. It is considered that toxicity of the excessive amount of chlorine in the effluent used in this research limits both the germination and the growth parameters.

The most unfavourable impact of the sodium overabundance (potassium and magnesium absorptions were adversely affected) was its inhibitive effect on calcium absorption [24, 21]. The lowest values of sulfur, aluminium, and calcium were obtained from the control group, while it was realized that the values increase as the amount of effluent increased in the irrigation water. In addition, it was found that copper and

potassium contents of the shoots were at the highest level in tap water and decreased as the amount of effluent increased in the irrigation water. No regular changes were detected in the other elements. Because of the complex interactions within the elements, it is so difficult to give remarkable results.

When elemental contents of the shoots irrigated with tap water and 1:10 diluted effluent were compared, it was found that copper, potassium, manganese, and zinc were at higher levels in tap water treatment, whereas carbon, sulfur, aluminium, calcium, chrome, iron, magnesium, and sodium were detected to be more in the shoots, irrigated with 1:10 diluted effluents.

High levels of calcium, magnesium, and sodium contents (1:10 dilutions) in rice shoots may indicate that the effect of chlorine overabundance was probably more dominant than that of sodium. However, further investigation is needed to proof this effect. On the other hand, decreases in the copper, manganese, and zinc absorption interactions were most probably caused by very high pH and calcium contents. Potassium content was higher in the tap water but lower in the effluents, which was most likely caused by high levels of chlorine and sodium in the effluents and in accordance with the data presented in the literature.

The data on some elemental contents in the roots of the seedlings irrigated with various dilutions of pretanning effluents and tap water were provided in Table 6.

Table 6

Some elemental contents of the roots [mg/kg]

| Element | Irrigation with tap water | Irrigation with pretanning effluent at dilution | | |
|---------|---------------------------|---|---------|---------|
| | | 1:80 | 1:40 | 1:10 |
| Al | 4513.4 | 4965.1 | 10435.0 | 8805.6 |
| Ca | 9234.6 | 10894.1 | 8361.3 | 9298.2 |
| Cr | 33.12 | 13.76 | 19.51 | 18.58 |
| Cu | 95.18 | 62.49 | 52.37 | 38.81 |
| Fe | 13275.9 | 20929.0 | 16738.4 | 14856.7 |
| K | 13775.2 | 12732.8 | 16120.3 | 13430.9 |
| Mg | 2622.5 | 3325.3 | 2740.0 | 3058.3 |
| Mn | 7332.1 | 7332.7 | 4131.9 | 2225.0 |
| Na | 3094.1 | 11607.5 | 6743.6 | 8393.6 |
| Zn | 406.76 | 334.10 | 153.58 | 85.41 |

When some elemental contents obtained from the analyses of the roots of rice were considered, it was detected that copper and zinc contents were at the highest level in the samples irrigated with tap water, while they decreased as the amount of effluent increased in the irrigation water (1:80, 1:40 and 1:10).

Aluminium, calcium, iron, magnesium, and sodium contents in roots irrigated with 1:10 diluted effluent were higher than in those irrigated with tap water. However, it

was found that chrome, copper, potassium, manganese, and zinc contents decreased in the roots irrigated with 1:10 dilution compared to tap water. Magnesium and sodium levels were lower in tap water compared to the three dilutions. Manganese, zinc, and copper contents decreased in accordance with the results obtained from the analyses of the shoots. The decrease in the potassium content was likely to be caused by the high levels of chlorine and sodium. High values observed in the elemental contents of the roots can be explained with the tendency for luxury consumption by plants.

Gupta and Sinha [22] determined the risk factors of the composite samples obtained from some plants in an area where treated tannery effluents were charged into. They observed the highest risk factor in rice in terms of chrome content. The researchers suggested that plant species with high risk factor of metal accumulation should not be grown in agricultural areas of similar characteristics and edible parts of the plants should be regularly monitored before consumption.

4. CONCLUSION

Leather production is an important income source in the developing countries as well as in Turkey. It is a sector that offers a lot of opportunities such as employment and large amount of foreign currency inflow. On the other hand, it is evident that it causes environmental pollutions such as unpleasant smells, noises, liquid, and solid waste materials. Effluents give harm not only to the place they are discharged into but also to other ecosystems. In the present study the effects of effluents resulting from pretanning processes (soaking, dehairing-liming, deliming-bating, degreasing and pickling) on the germination and seedling growth of rice, it was determined that undiluted effluent (1:0) completely inhibited germination and that other dilutions (1:10, 1:40, 1:80) slowed down germination and seedling growth. It was pointed out by other researchers that considerable changes as, high salinity, pH changes, heavy metal accumulation, etc. caused by the pollutants in the tannery effluents are not only in germination and morphological properties of the plants but also in the edible parts consumed by humans and other living organisms.

The effluents should be completely treated before being discharged into the environment, and they should not be used for irrigation. In addition, products cultivated in and around fields where tanneries are located should be periodically and carefully monitored and analysed against the possible contamination risks even if the effluents have been safely treated. This study, as a preliminary investigation, would provide guidance for further in-situ researches on rice which requires ample water. It is notable that increasing environmental awareness in the public as well as the importance of the environmental issues of Turkey and European Union has brought the subject into prominence.

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