

Tradename: AC Det'Ox Hair

Code: 21030

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Test Performed:

Tensile Strength After Repeated Washes Assay

Introduction

Hair plays a crucial role in personal appearance, social identity, and overall well-being. The mechanical integrity of hair is a key determinant of perceived hair health, influencing resilience to breakage, manageability, and styling performance. Among the many external stressors that influence hair fiber structure and function, repeated washing is an underestimated contributor to cumulative hair damage. Despite the benefits of shampoo and conditioner on physical appearance and scalp hygiene, repeated washing without strengthening and protective ingredients exert a deleterious effect on the hair shaft.

Specifically, frequent wetting and drying cycles weaken the protective lipid and protein layers of cuticles as the hair shaft repeatedly expands and contracts. These fluctuations in hair shaft size led to increased porosity, moisture imbalance, and surface roughness which deteriorates the structural and functional integrity of hair. Moreover, washing with hard water can further compound the damage as heavy metals deposit on the hair fiber. The resultant diminished tensile strength and increased susceptibility to mechanical stress and breakage of hair fibers can be exacerbated by environmental insult, surfactant exposure, and physical manipulation (e.g., towel drying, combing while wet, heat styling). This is particularly evident in hair types with pre-existing structural vulnerabilities, such as chemically treated, curly, or textured hair. Therefore, cosmetic ingredients designed to reinforce and promote healthier hair, especially in shampoo and conditioner formulations, are critical.

Accordingly, an *ex vivo* Tensile Strength After Repeated Washes Assay was conducted to assess the ability of **AC Det'Ox Hair** to protect and strengthen hair after repeated exposure to heavy metals and washings.

Assay Principle

Virgin human hair tresses were repeatedly exposed to a heavy metal solution then washed with shampoo and conditioner to understand the protective and strengthening capability of an added test article. After treatment, individual fibers were assessed to determine hair strength and resistance to deformation. Tensile properties are assessed by applying force to individual fibers until each strand breaks and force to break, tenacity, percent elongation, and elasticity are calculated. Force to break and tenacity measure hair fiber strength with higher values indicating a stronger and healthier fiber. Force to break is the maximum force required to break a fiber and tenacity reveals the breaking strength with respect to fiber linear density. Percent elongation and elasticity indicate ductility and resistance to deformation. Percent elongation provides fiber deformation before breaking and is inversely related to elasticity which expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber length. More fiber elongation indicates more damage, whereas greater breaking stress indicates more elasticity.

Materials

- A. **Hair Samples:** Assorted Human Virgin Hair Tresses
- B. **Products:** Base Shampoo and Conditioner (Table 1); Heavy Metal Solution (Table 2)
- C. **Equipment:** Instron (Method ASTM D3822)
- D. **Software:** Excel Analysis ToolPak (Microsoft)

Table 1. Base Shampoo and Base Conditioner INCI's and Compositional Breakdowns.

<u>Base Shampoo Formulation</u>	<u>%</u>	<u>Base Conditioner Formulation</u>	<u>%</u>
Water	41.0	Water	76.0
Guar Hydroxypropyltrimonium Chloride	1.0	Polyquaternium-10	1.0
Sodium Methyl 2-Sulfolaurate (and) Disodium 2-Sulfolaurate	35.0	Glycerin	3.0
Cocamidopropyl Betaine	15.0	Water & Centrimonium Chloride	2.0
Lactobacillus Ferment & Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	4.0	Behentrimonium Methosulfate & Cetearyl Alcohol & Butylene Glycol	8.0
Polysorbate 20	2.0	Hydrogenated Ethylhexyl Oliviate (and) Hydrogenated Olive Oil Unsaponifiables	5.0
Fragrance	2.0	Lactobacillus Ferment	4.0
		Fragrance	1.0

Table 2. Heavy Metal Elements and Concentrations.

<u>Element</u>	<u>Concentration (ppm)</u>
Lead	0.1
Cadmium	0.05
Manganese	3.0
Chromium	1.0
Nickel	1.0
Zinc	50
Copper	13
Fluoride	40
Selenium	0.5
Thallium	0.02
Calcium	500

Methods

Sixteen virgin hair tresses were collected and classified using the Andre Walker Hair Typing System (Image 1). Tresses from each hair type were assigned to the conditions described in Table 3. For each hair type, three tresses were soaked for thirty minutes in a heavy metal solution (Table 2). After soaking, one tress was rinsed with water as the Heavy Metal Control, one tress received Base Shampoo and Conditioner, and the third received 2.0% **AC Det'Ox Hair** in Base Shampoo and Conditioner. All treatments were thoroughly massaged into the hair for 60 seconds before rinsing. After conditioners were rinsed out, the tresses were allowed to fully air-dry. Tresses were exposed to heavy metals followed by washes with their designated treatments seven times. An untreated tress was left unaltered as the Untreated Control for each hair type.

Image 1. Andre Walker Hair Typing System

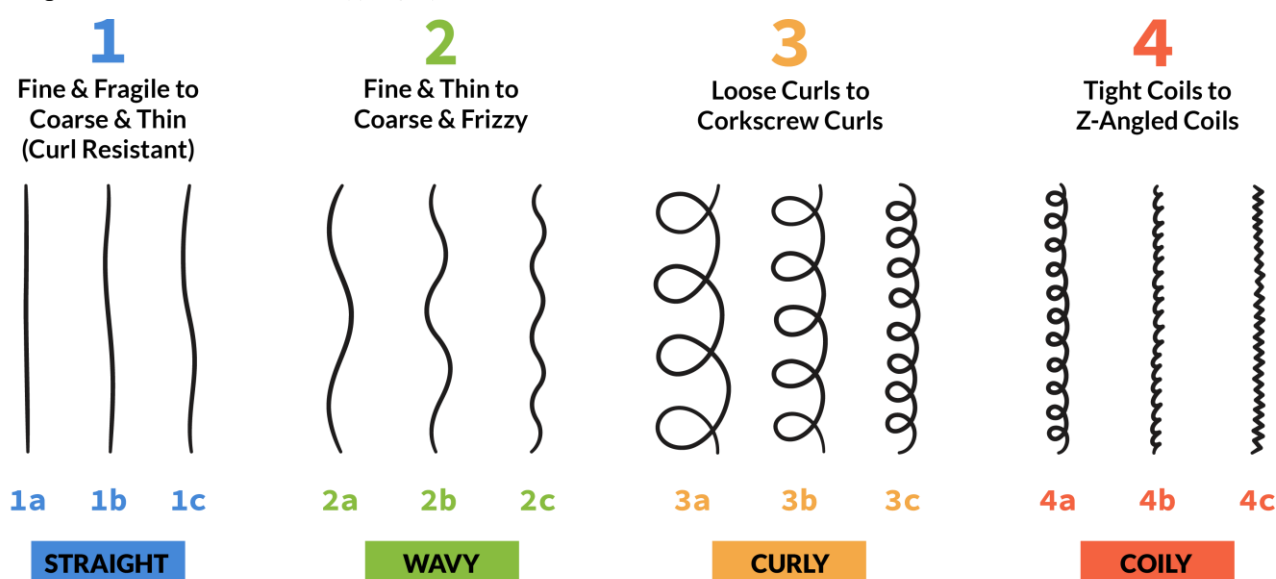


Table 3. Descriptions of the Conditions for each Hair Type.

Condition	Treatment Description	Hair Type
Untreated Control	No Metal Exposure, No Washes	1C-Straight-Black 3B-Curly-Black 1B-Straight-Blonde 2B-Wavy-Brunette
Heavy Metal Control	7 Metal Exposures, 7 Water Rinses	
Base Shampoo and Conditioner	7 Metal Exposures, 7 Washes with Base Shampoo and Conditioner	
2.0% AC Det'Ox Hair	7 Metal Exposures, 7 Washes with 2.0% AC Det'Ox Hair in Base Shampoo and Conditioner	

The Instron method ASTM D3822 was used to evaluate the effect of **AC Det'Ox Hair** on the integral structure and properties of the hair after repeated heavy metal exposure and washing. Single fiber testing was performed to assess the strength of the hair in terms of force to break, tenacity, percent elongation, and Modulus of Elasticity. 25 hair strands from each tress were tested and average values were recorded. One-way ANOVAs were performed between groups with statistical significance accepted at $p \leq 0.05$.

Results

The data obtained met criteria for a valid assay as the controls performed as anticipated. For all hair types, hair tresses exposed seven times to the Heavy Metal Solution demonstrated a reduction in all tensile properties tested compared to the Untreated Controls. Similarly, all tresses washed seven times with the Base Shampoo and Conditioner after metal exposure demonstrated a reduction in all tensile properties tested compared to the Untreated Controls. Conversely, all hair types washed seven times with 2.0% AC Det'Ox Hair demonstrated improvements in all tensile properties compared to the Heavy Metal Control and hair repeatedly washed with the Base Shampoo and Conditioner.

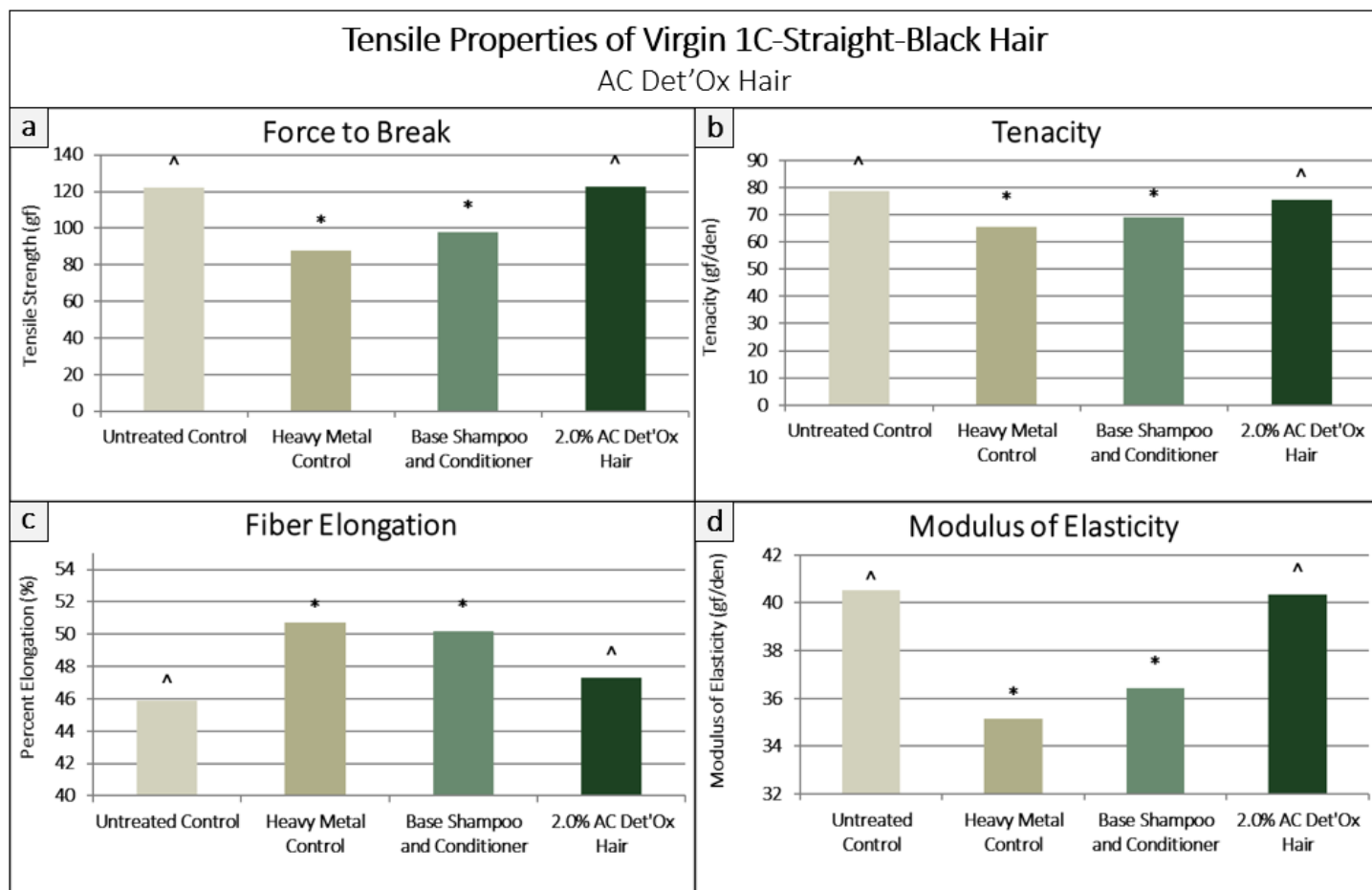


Figure 1. Average Tensile Properties of 1C-Straight-Black Tresses After Seven Washes. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal Control. a) Force to Break gives the maximum force required to break each fiber where an increase indicates fiber protection, and a decrease indicates less protection. b) Tenacity gives the break strength of each fiber where an increase indicates fiber protection, and a decrease indicates less protection. c) Fiber Elongation illustrates fiber deformation before breaking where lower values indicate stronger hair fibers and higher values indicate more damaged fibers. d) Modulus of Elasticity expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber where an increase indicates stronger hair fibers, and a decrease indicates more damaged fibers.

Table 4. P-values from one-way ANOVA between Conditions for 1C-Straight-Black Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal Control.

		Heavy Metal Control	Base Shampoo and Conditioner	2.0% AC Det'Ox Hair
Force to Break	Untreated Control	< 0.001*	0.004*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.001^
Tenacity	Untreated Control	0.004*	0.032*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.009^
Fiber Elongation	Untreated Control	0.002*	0.003*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.043^
Modulus of Elasticity	Untreated Control	0.032*	0.045*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.031^

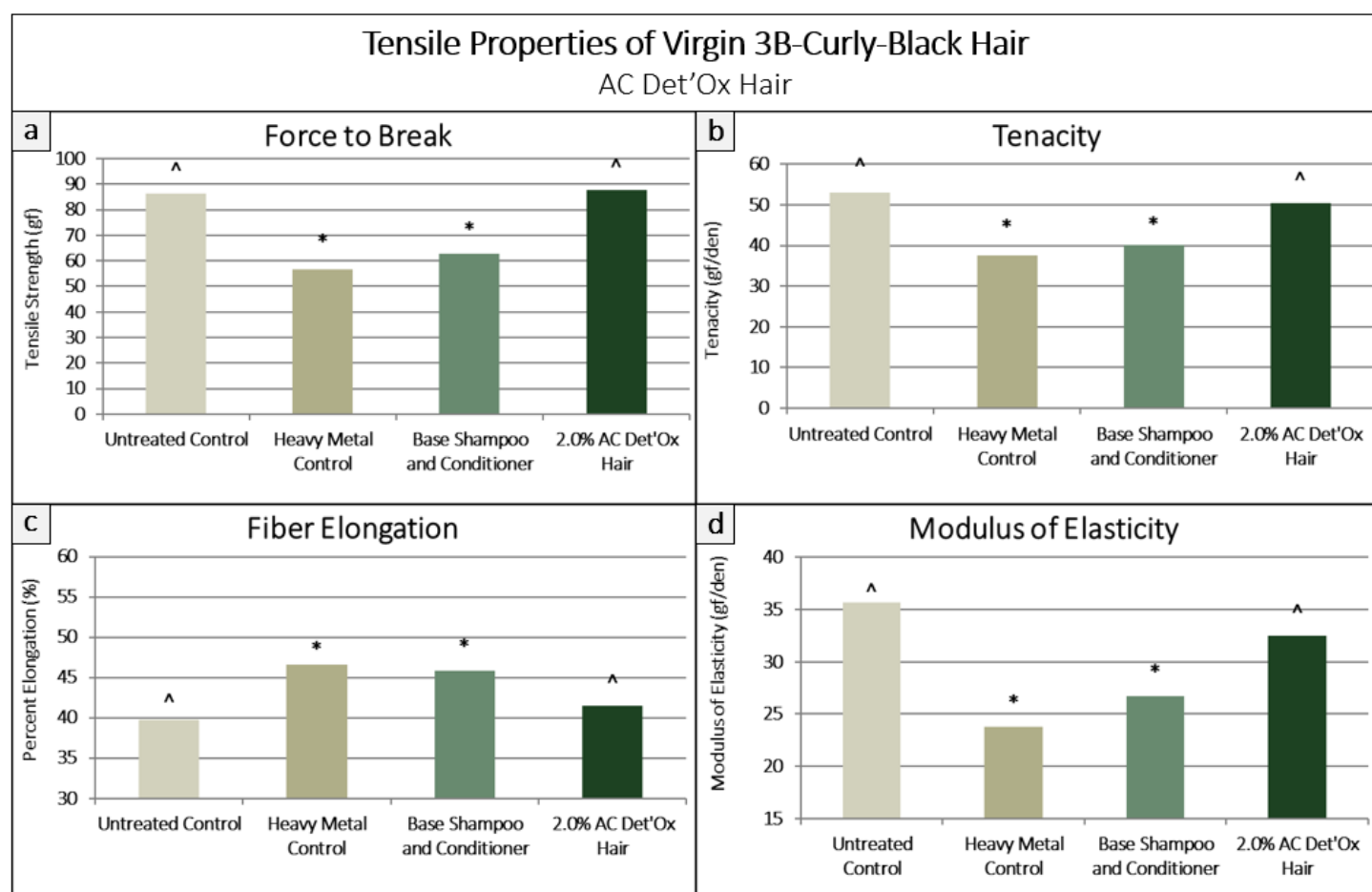


Figure 2. Average Tensile Properties of 3B-Curly-Black Hair Tresses After Seven Washes. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal Control. a) Force to Break gives the maximum force required to break each fiber where an increase indicates fiber protection, and a decrease indicates less protection. b) Tenacity gives the break strength of each fiber where an increase indicates fiber protection, and a decrease indicates less protection. c) Fiber Elongation illustrates fiber deformation before breaking where lower values indicate stronger hair fibers and higher values indicate more damaged fibers. d) Modulus of Elasticity expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber where an increase indicates stronger hair fibers, and a decrease indicates more damaged fibers.

Table 5. P-values from one-way ANOVA between Conditions for 3B-Curly-Black Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal Control.

		Heavy Metal Control	Base Shampoo and Conditioner	2.0% AC Det'Ox Hair
Force to Break	Untreated Control	< 0.001*	0.005*	> 0.05
	Heavy Metal Control	-----	> 0.05	< 0.001^
Tenacity	Untreated Control	0.002*	0.021*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.004^
Fiber Elongation	Untreated Control	0.001*	0.003*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.045^
Modulus of Elasticity	Untreated Control	0.001*	0.022*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.006^

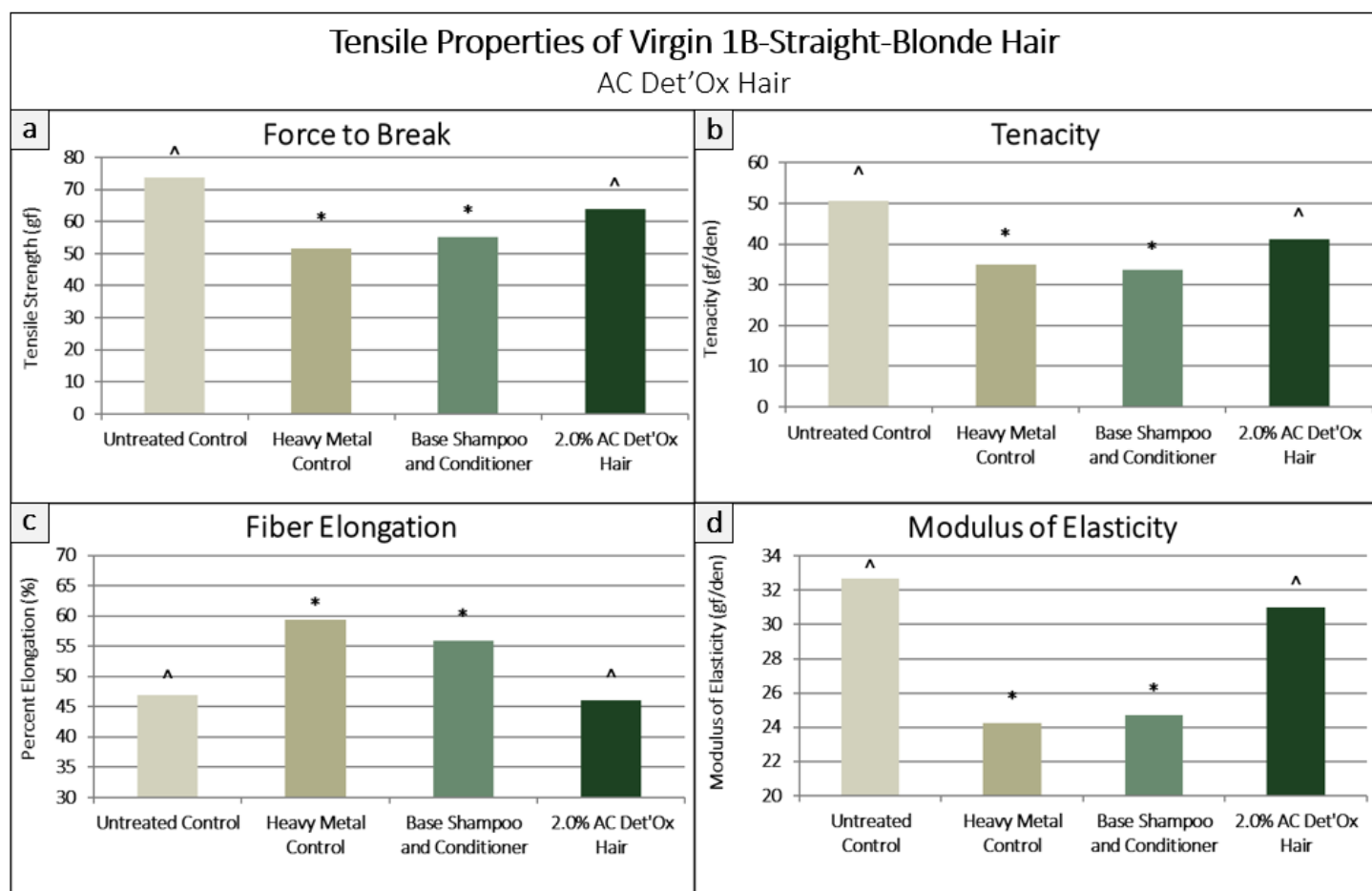


Figure 3. Average Tensile Properties of 1B-Straight-Blonde Hair Tresses After Seven Washes. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal Control. a) Force to Break gives the maximum force required to break each fiber where an increase indicates fiber protection, and a decrease indicates less protection. b) Tenacity gives the break strength of each fiber where an increase indicates fiber protection, and a decrease indicates less protection. c) Fiber Elongation illustrates fiber deformation before breaking where lower values indicate stronger hair fibers and higher values indicate more damaged fibers. d) Modulus of Elasticity expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber where an increase indicates stronger hair fibers, and a decrease indicates more damaged fibers.

Table 6. P-values from one-way ANOVA between Conditions for 1B-Straight-Blonde Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal Control.

		Heavy Metal Control	Base Shampoo and Conditioner	2.0% AC Det'Ox Hair
Force to Break	Untreated Control	0.041*	0.035*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.042^
Tenacity	Untreated Control	0.028*	0.028*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.009^
Fiber Elongation	Untreated Control	0.001*	0.008*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.034^
Modulus of Elasticity	Untreated Control	0.036*	0.043*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.036^

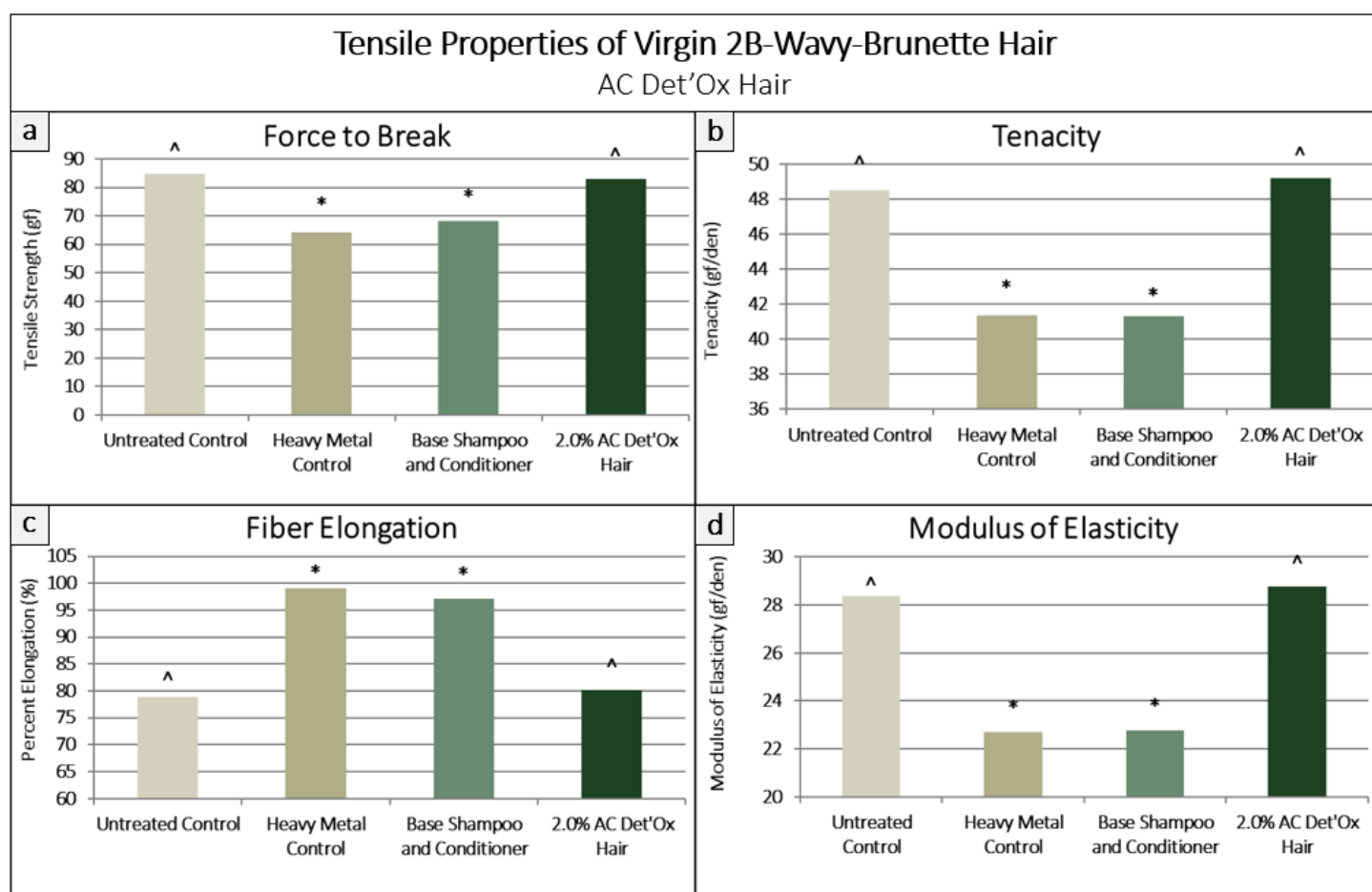


Figure 4. Average Tensile Properties of 2B-Wavy-Brunette Hair Tresses After Seven Washes. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal Control. a) Force to Break gives the maximum force required to break each fiber where an increase indicates fiber protection, and a decrease indicates less protection. b) Tenacity gives the break strength of each fiber where an increase indicates fiber protection, and a decrease indicates less protection. c) Fiber Elongation illustrates fiber deformation before breaking where lower values indicate stronger hair fibers and higher values indicate more damaged fibers. d) Modulus of Elasticity expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber where an increase indicates stronger hair fibers, and a decrease indicates more damaged fibers.

Table 7. P-values from one-way ANOVA between Conditions for 2B-Wavy-Brunette Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal Control.

		Heavy Metal Control	Base Shampoo and Conditioner	2.0% AC Det'Ox Hair
Force to Break	Untreated Control	0.019*	0.029*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.015^
Tenacity	Untreated Control	0.034*	0.027*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.004^
Fiber Elongation	Untreated Control	0.040*	0.018*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.010^
Modulus of Elasticity	Untreated Control	0.019*	0.024*	> 0.05
	Heavy Metal Control	-----	> 0.05	0.042^

Discussion

As demonstrated in Figures 1 – 4, repeated exposure to Heavy Metals negatively impacts the tensile properties of 1C-Straight-Black, 3B-Curly-Black, 1B-Straight-Blonde, and 2B-Wavy-Brunette hair tresses. Moreover, washing with Base Shampoo and Conditioner alone does not protect against the negative impacts of Heavy Metals. Together, this data demonstrates repeated heavy metal exposure and hair washing cause significant damage to diverse hair types by degrading the tensile properties of individual fibers.

With respect to 1C-Straight-Black hair, incorporating 2.0% **AC Det'Ox Hair** into shampoo and conditioner formulations protected the tensile properties after repeated heavy metal exposure and washes. Specifically, after seven metal exposures, the Heavy Metal Control tresses demonstrated 28%, 17%, and 13% reductions in force to break, tenacity, and elasticity, respectively, and experienced an 11% increase in fiber elongation compared to the Untreated Control. Similarly, tresses washed with the Base Shampoo and Conditioner after metal exposure demonstrated 20%, 12%, and 10% reductions in force to break, tenacity, and elasticity, respectively, and fiber elongation increased by 9% compared to the Untreated Control (Figure 1; Table 4). Conversely, tresses exposed to heavy metals then treated seven times with 2.0% **AC Det'Ox Hair** in Base Shampoo and Conditioner maintained similar force to break, tenacity, fiber elongation, and elasticity compared to the Untreated Control (Figure 1; Table 4). Furthermore, 2.0% **AC Det'Ox Hair** elicited 40%, 15%, and 15% increases in force to break, tenacity, and elasticity, respectively, and improved fiber elongation by 7% after seven metal exposures and washes compared to the Heavy Metal Control (Figure 1; Table 4). Collectively, this data indicates **AC Det'Ox Hair** protects and strengthens 1C-Straight-Black hair after repeated exposure to heavy metals and washes.

Regarding 3B-Curly-Black hair, incorporating 2.0% **AC Det'Ox Hair** into shampoo and conditioner formulations protected the tensile properties after repeated heavy metal exposure and washes. Specifically, after seven metal exposures, the Heavy Metal Control tresses demonstrated 34%, 29%, and 33% reductions in force to break, tenacity, and elasticity, respectively, and experienced a 17% increase in fiber elongation compared to the Untreated Control. Similarly, tresses washed with the Base Shampoo and Conditioner after metal exposure demonstrated 27%, 24%, and 25% reductions in force to break, tenacity, and elasticity, respectively, and fiber elongation increased by 15% compared to the Untreated Control (Figure 2; Table 5). Conversely, tresses exposed to heavy metals then treated seven times with 2.0% **AC Det'Ox Hair** in Base Shampoo and Conditioner maintained similar force to break, tenacity, fiber elongation, and elasticity compared to the Untreated Control (Figure 2; Table 5). Furthermore, 2.0% **AC Det'Ox Hair** elicited 55%, 34%, and 37% increases in force to break, tenacity, and elasticity, respectively, and improved fiber elongation by 11% after seven metal exposures and washes compared to the Heavy Metal Control (Figure 2; Table 5). Together, this data indicates **AC Det'Ox Hair** protects and strengthens 3B-Curly-Black hair after repeated exposure to heavy metals and washes.

In terms of 1B-Straight-Blonde hair, incorporating 2.0% **AC Det'Ox Hair** into shampoo and conditioner formulations protected the tensile properties after repeated heavy metal exposure and washes. Specifically, after seven metal exposures, the Heavy Metal Control tresses demonstrated 30%, 31%, and 26% reductions in force to break, tenacity, and elasticity, respectively, and experienced a 27% increase in fiber elongation compared to the Untreated Control. Similarly, tresses washed with the Base Shampoo and Conditioner after metal exposure demonstrated 25%, 33%, and 24% reductions in force to break, tenacity, and elasticity, respectively, and fiber elongation increased by 19% compared to the Untreated Control (Figure 3; Table 6). Conversely, tresses exposed to heavy metals then treated seven times with 2.0% **AC Det'Ox Hair** in Base Shampoo and Conditioner maintained similar force to break, tenacity, fiber elongation, and elasticity compared to the Untreated Control (Figure 3; Table 6). Furthermore, 2.0% **AC Det'Ox Hair** elicited 23%, 17%, and 28% increases in force to break, tenacity, and elasticity, respectively, and improved fiber elongation by 22% after seven metal exposures and washes compared to the Heavy Metal Control (Figure 3; Table 6). Together, this data indicates **AC Det'Ox Hair** protects and strengthens 1B-Straight-Blonde hair after repeated exposure to heavy metals and washes.

Lastly, incorporating 2.0% **AC Det'Ox Hair** into shampoo and conditioner formulations enhances the tensile properties of 2B-Wavy-Brunette hair after repeated heavy metal exposure and washes. Specifically, after seven metal exposures, the Heavy Metal Control tresses demonstrated 24%, 15%, and 20% reductions in force to break, tenacity, and elasticity, respectively, and experienced a 26% increase in fiber elongation compared to the Untreated Control. Similarly, tresses washed with the Base Shampoo and Conditioner after metal exposure demonstrated 20%, 15%, and 20% reductions in force to break, tenacity, and elasticity, respectively, and fiber elongation increased by 23% compared to the Untreated Control (Figure 4; Table 7). Conversely, tresses exposed to heavy metals then treated seven times with 2.0% **AC Det'Ox Hair** in Base Shampoo and Conditioner maintained similar force to break, tenacity, fiber elongation, and elasticity compared to the Untreated Control (Figure 4; Table 7). Furthermore, 2.0% **AC Det'Ox Hair** elicited 29%, 19%, and 27% increases in force to break, tenacity, and elasticity, respectively, and improved fiber elongation by 19% after seven metal exposures and washes compared to the Heavy Metal Control (Figure 4; Table 7). Collectively, this data indicates **AC Det'Ox Hair** protects and strengthens 2B-Wavy-Brunette hair after repeated exposure to heavy metals and washes.

Taken together, these results demonstrate **AC Det'Ox Hair** augments the tensile properties of 1C-Straight-Black, 3B-Curly-Black, 1B-Straight-Blonde, and 2B-Wavy-Brunette hair after repeated heavy metal exposure and washing when added to shampoo and conditioner formulations at recommended use-levels. In summary, **AC Det'Ox Hair** protects and strengthens diverse hair types from the damaging effects of consistent exposure to hard water.